London South Bank University

Study of Inclusion in Phase Inversion Emulsification and Multiple Emulsions

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To my loving family: father, mother, brothers, sisters, husband, and daughters.

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ABSTRACT

The phenomenon of 'inclusion' in emulsions refers to the inclusion of continuous phase into the dispersed phase drops and formation of multiple drops due to the tendency of the inner phase to stabilise the outer phase. Inclusion plays an important role in both catastrophic phase inversion (CPI) emulsification process and preparation of multiple emulsions. In CPI, inclusion leads to formation of abnormal multiple drops, which eventually invert to the opposite desired normal emulsion. On the other hand, in multiple emulsions, inclusion may have an adverse effect leading to instability of the multiple emulsions because of coalescence of inner drops, and/or coalescence of multiple drops, or even bursting of multiple drops. All these result in instability of multiple emulsions and accelerate release of the active agent. In the first part of this work, we carried out a comprehensive study on the rate and extent of inclusion in CPI emulsification at different formulation and process conditions for the first time. We also investigated the conditions under which the CPI emulsification method produces finer emulsions than the direct emulsification method, which means applying less energy could result in a finer emulsion. In the second part of this work, we investigated the conditions under which inclusion can be ignored during the preparation of multiple emulsions. We also carried out an extensive study on the optimisation of the process conditions to achieve maximum encapsulation efficiency in freshly prepared multiple emulsions.

In the last part of this work, we briefly looked at the possibility of synthesis of macroporous alginate hydrogel particles via CPI and through inclusion of an oil into sodium alginate drops but without success. However, we introduced a new modified emulsification process based on addition of calcium chloride water-in-oil emulsion to sodium alginate emulsion, which produced promising results.

The main results of each part of this research are introduced below.

- **Catastrophic Phase Inversion**

Cyclohexane, water and two non-ionic surfactants (Igepal CO-720 with HLB=14.2 and Igepal CO–520 with HLB=10) have been used to produce O/W emulsions via CPI method. Several series of experiments have been carried out at different phase fractions, surfactant concentration and affinity (HLB), stirring speed, and temperature to produce oil-in-water emulsions. The location of surfactant (oil or water phase) was also altered. The variations in conductivity were monitored to

observe the dynamic/extent of inclusion and the inversion point (time). Microscopic images of the emulsions have been acquired using optical microscopy, and the average drop size $D_{3,2}$ was measured.

The rate and extent of inclusion as the main parameter of catastrophic phase inversion (CPI) were studied at different conditions. The effects of different process and formulation parameters on the inversion time, the effective dispersed phase fraction, as well as the Sauter mean diameter of drops in the final emulsions, were also studied.

In most cases, the more inclusion the longer was the inversion time and the smaller the final drop size. Our results showed that both the effective dispersed phase fraction (f_d) and the internal phase fraction (Ø) gradually increased with relative time until phase inversion occured. This is due to inclusion of the continuous phase inside the dispersed phase and the formation of multiple drops. It was also found that at higher phase fraction f_w, inversion occurred almost immediately with no significant inclusion. Moreover, the results showed that more inclusion occurred at higher HLB than lower HLB.

The results revealed that the rate of inclusion was faster as stirring speed decreased. It was also found that inversion time and drop size were strongly affected by stirring speeds. At high HLB of 14, the inversion time showed a maximum value when intermediate range of stirring speed was used. While for a mixture of surfactants (HLB=13), the inversion time decreased with increasing stirring speeds. At low stirring speeds, CPI is capable of producing finer emulsions than the direct method, i.e. less energy input.

The extent of inclusion decreased as temperature increased. CPI is more efficient in producing finer drops at high temperatures than at low temperatures, with a shorter time for the emulsion to invert. In comparison to the direct method, CPI produces finer emulsions at lower temperatures.

CPI emulsification is a superior method in comparison to direct emulsification for producing fine emulsions, especially when extensive inclusion occurs leading to the formation of smaller inner droplets (multiple drops), which in turn form the dispersed phase in the inverted emulsion, and when the surfactant(s) is (are) dissolved in the oil phase.

❖ **Multiple Emulsions**

The second part of this research focuses on the formation of multiple emulsions by a two-step emulsification method. In this work, different formulations have been used to prepare stable emulsions, water-in-oil-in-water emulsions (water-in-paraffin-in-water), in the presence of non-ionic surfactants of different hydrophilicity. To evaluate the stability of these formulations, the changes in emulsion conductivities were monitored in order to calculate encapsulation efficiency; the drop size was measured and an optical microscopy was used to observe the drop morphology. The effects of different parameters on the Sauter mean diameter $D_{3,2}$ of drops in the final emulsions have been investigated. The results presented in this work reveal that inclusion is not important during preparation of multiple emulsions. It was found that the droplet exit process was the dominant release mechanism. We successfully predicted the encapsulation efficiency for multiple emulsions from the conductivity data by modifying Brugmann's law which was originally introduced for single emulsions.

It was found that increasing the primary phase fraction of multiple emulsions led to a reduction in the drop size of the final emulsions and hence to a reduction in the encapsulation efficiency. Maximum encapsulation efficiency with larger drop size was obtained using 0.1M of NaCl while a maximum drop size was obtained when intermediate values of the secondary phase fraction were used. Applying high shear rates during primary emulsification led to an increase in the encapsulation efficiency. The use of the right amount of the surfactant in both phases allowed the production of multiple emulsions of high encapsulation. An increase in the oil viscosity was found to alter the encapsulation efficiency.

❖ **Alginate Hydrogel Particles**

In this research, a new modified emulsification method was employed for the synthesis of macroporous hydrogel particles. A series of experiments were carried out using sodium alginate (NaAlg), deionised water, an oil soluble surfactant (Span 20) and $CaCl_2$ as a crosslinker. First two normal water-in-oil (W/O) emulsions were prepared by adding NaAlg and $CaCl_2$ aqueous solutions to an oil phase containing Span 20. In a second step, the two emulsion were mixed for 2 hours. The experiments were conducted at 25^{o}C. Generally, semi-solid large irregular-shaped hydrogel particles were formed which were then washed, dried and analysed by scanning electron microscopy (SEM). A mechanism was

suggested for the production of large polymer matrices through coalescence of NaAlg and $CaCl_2$ drops. The effect of NaAlg and $CaCl_2$ concentrations on the hydrogel particles size were briefly investigated. Stable hydrogel particles with high water absorption were obtained at low NaAlg concentration and intermediate $CaCl_2$ concentration. Interestingly, particles with a narrow distribution size were produced. These results are preliminary and more studies are required to verify the credibility of this new method.

INTRODUCTION

Motivation

Despite the numerous research publications on emulsions and phase inversion emulsification processes and their applications such as synthesis of multiple emulsions, there is still a need to explore the underlying mechanisms and optimum conditions to produce fine and stable emulsions. Here a brief background and motivations for carrying out this research are outlined.

- **Phase inversion emulsification**

For many years, emulsions have been a popular subject for researchers (Walstra, 1993; Israelachvili, 1994; Tadros, 1993; Derkach, 2009; Tzoumaki et al., 2011). Emulsions are used in a variety of industrial products such as paint, cosmetics, food and pharmaceuticals (Perazzo et al., 2015; Lawrence and Rees, 2012; Fanun, 2012; Bilbao-Sainz, 2010; Bibette and Leal-Calderon, 1996; Chappat, 1994; Forgiarini et al., 2001; Lissant, 1974; Tadros, 1994). Emulsion and microemulsions systems are also used in enhanced oil recovery and other areas of the gas and oil industry (Groeneweg et al., 1998; Acharya and Hartley, 2012).

Phase inversion emulsification method is a common low-energy route to make stable emulsions. Catastrophic phase inversion is one of the most important aspects of emulsion science; it is the preferred method because it allows for the formation of a smaller drop size than conventional emulsification process, with a lower energy input (Perazzo et al., 2015). Due to the variety of applications related to this process and the complexity of the problem, most of the publications in this area have focused either on the phase behaviour, interfacial properties and/or mixing process of the two phases to produce fine emulsions (stable emulsion) (Brooks and Richmond, 1994a; Vaessen et al., 1996; Peña and Salager, 2001; Bouchama et al., 2003; Sajjadi et al., 2002b, 2006a, 2006b; Mira et al., 2003; Salager et al., 2004; Rondón-González et al., 2006a, 2007; Thakur et al., 2008; Jahanzad et al., 2009; Campbell et al., 2012; Liu et al., 2012; Zang and Clegg, 2013; Lv et al., 2014). One of the most important properties of an emulsion is its average drop size as this greatly influences the characteristics of the emulsion, for example, the rheology and stability of an emulsion. Although all these features are quite important in studying phase inversion and consequently many research works have been undertaken in this area, a comprehensive

picture is still lacking, and more investigation is required. In particular, the mechanisms governing the inversion phenomenon have not been completely elucidated upon, and quantitative predictions of the phase inversion point are limited to specific systems and experimental conditions. In light of recent research interest in emulsion formation, there is a need to expand the current body of knowledge regarding the mechanism of catastrophic phase inversion and role of different processes and formulation parameters on formation of fine emulsions. By reviewing different approaches, this project presents a thorough study of inclusion as the mechanism of catastrophic phase inversion and the possible reasons for the formation of fine drops through catastrophic phase inversion in comparison to direct emulsification method.

- **Multiple emulsions**

Multiple emulsions were first reported in 1925 by Seifriz (Matos et al., 2014). Multiple emulsions are also called double emulsions, and they are ternary systems, having either a water-in-oil-in-water (W/O/W) or an oil-in-water-in-oil (O/W/O) assembly. Due to their structural properties, multiple emulsions can entrap necessary substances in the internal droplets. They are used in applications where controlled (slow and prolonged) release of active materials from the internal to the external phase is desired. For instance, many food products undergo an undesirable loss of quality due to the rapid release of unstable flavour compounds during processing or storage (Dickinson et al., 1994). One way to overcome this problem is to utilize a multiple emulsion with the flavour compound trapped in the internal droplets (Gaonkar, 1994). For this reason multiple emulsions can be found in a number of applications in many fields in the industry such as; medicine, pharmacy, cosmetics, food as well as separation processes (Garti and Bisperink, 1998; Garti, 1997b; Garti and Aserin, 1996; Garti and Lutz, 2004; Zhu et al., 2017; Sapei et al., 2012; Schmidts et al., 2010).

Water-in-oil-in-water (W/O/W) multiple emulsions consist of two emulsions; a water-in-oil (W/O) emulsion and an oil-in-water (O/W) emulsion (Sapei et al., 2012; Schmidts et al., 2010). Due to the presence of two aqueous domains separated by an oil layer, the inner aqueous phase offers great potential for the encapsulation and controlled release of hydrophilic bioactive ingredients (Sapei et al., 2012). For this reason, W/O/W multiple emulsions have potential

applications in food, cosmetic and pharmaceutical industries. They act as vehicles/transporters for encapsulation and delivery of nutrients during food digestion or drug release (Garti, 1997b; Spyropoulos et al., 2011). Thus making it possible to mask taste or smell; protect against oxidation, light, or enzymatic degradation; or to ensure the controlled release of the active ingredients under the effect of dilution, shear, or agitation (Kanouni et al., 2002). In food applications water-in-oil-in-water (W/O/W) multiple emulsions are the most commonly studied multiple emulsions because most food emulsions have a continuous aqueous phase and there is a greater availability/selection of food grade hydrophilic emulsifiers that can be used to stabilize the dispersed oil phase (O'Regan and Mulvihill, 2010).

The major problem that limits the applications of multiple emulsions is that they are thermodynamically unstable, due to the excess of free energy associated with the surface of the emulsion droplets (Garti, 1997a). One of the biggest problems is creaming which could result at the end of coalescence and separation mainly due to inclusion (Garti and Aserin, 1996; Benna et al., 2008; Leal-Calderon et al., 2012). Therefore, the emulsification processes and the formulation variables are needed to be rightly chosen to produce stable multiple emulsions with minimum release or high encapsulation efficiency. However, in many scenarios, it is hard to eliminate release during emulsification, which often leads to low encapsulation efficiency. Despite the efforts made to improve the tendency for droplet coalescence, the instability of multiple emulsions is still a major unsolved problem.

In light of recent research interest in multiple emulsions formation, there is still need for more experimental investigations to improve the stability of multiple emulsions. This study aims to investigate the role of inclusion in instability of multiple emulsions, and conditions under which inclusion can be ignored. Also the effects of different formulations and process variables on the stability of freshly-prepared W/O/W emulsions. Stability was assessed by monitoring encapsulation efficiency during emulsification process.

- **Hydrogel Particles**

Hydrogels have received considerable attention for many years due to their potential use in different applications (Leong et al., 2016; Jabeen et al., 2015; Stocke et al., 2015; Susan et al., 2012; Samchenko et al., 2011; Bhattarai et al.,

2010, Hamidi et al., 2008; Lin and Metters, 2006; Peppas et al., 2000; Peppas and Khare, 1993; Graham and McNeill, 1984). Due to their chemical, three-dimensional (3D), unique physical properties, mechanical properties, highly porous structure, higher permeability to small molecules and release of entrapped molecules in a controlled manner, hydrogels have received a great attention of exploration in many applications such as biomedical engineering, pharmaceutical, and biomaterials science. They also possess a degree of flexibility very similar to natural tissue due to their large water content (Ahmed, 2015).

Sodium alginate has shown excellent water solubility. It can create anionic cross-linked hydrogel under mild conditions (Zhao et al., 2015). The hydrogels market in Europe is driven by the growing demand for sodium alginate in various industrial applications, particularly the food and pharmaceutical industries. In the past two decades, sodium alginate has been one of the most popular encapsulation materials for controlled delivery applications. Sodium alginate can form hydrogel easily and safely upon the addition of cations (Voo et al., 2016). Extrusion and emulsification methods have been the most common methods to produce alginate hydrogel particles of different sizes and morphologies to suit the needs of different applications (Burkert et al., 2007, Chauhan et al., 2012).

In this research, in line with the rest of the work, first we explore the possibility of inclusion of oil in to sodium alginate drops to produce hydrogel particles with large pores, which are suitable for tissue engineering and other applications with high water content requirement. Also a new modified emulsification method, based on mixing of emulsions of sodium alginate and calcium chloride, to produce macroporous hydrogel particles is introduced and investigated.

REASEARCH AIM AND OBJECTIVES

- **Catastrophic Phase Inversion Emulsification (CPI)**

A comprehensive study of inclusion in catastrophic phase inversion (CPI) is carried out in this work for the first time. The aim is to study the inclusion as underlying mechanism of CPI and its effect on dynamic of phase inversion and drop size of the final emulsion. The objectives are to study the effects of important formulation and process variables; (i) phase fraction, (ii) surfactant concentration and affinity (HLB) and location, (iii) stirring speed and (iv) temperature; on the rate and extent of inclusion, inversion time, and final drop

size, and also to (v) compare the CPI and direct emulsification methods with respect to their capability to produce fine emulsions.

Methodology: This research focuses on the emulsification processes using a model oil/water emulsion (cyclohexane/water). Emulsions are stabilised using a single or mixture of non-ionic surfactants (Igepal CO-720 of HLB14.2; Igepal CO–520 of HLB10). Microscopic images of the emulsions are acquired using optical microscopy and the average droplet size is measured using Mastersizer. Variation in conductivity of emulsion is monitored to observe the rate/extent of inclusion and inversion time.

- **Multiple Emulsions**

The aims of the study of multiple emulsions are, for the first time, to investigate the dominant mechanism of instabilisation and release, and also important of inclusion vs. other exit mechanisms, during the preparation of multiple emulsions, and also to find the optimum conditions for achieving maximum encapsulation efficiency in freshly-prepared multiple emulsions. It is also aimed to derive a simple correlation to predict the encapsulation efficiency in multiple emulsions from conductivity measurements.

The objectives are to (i) evaluate the importance of different release mechanisms during secondary emulsification (i.e., preparation of multiple emulsions), oulining the dominant mechanism, and also study the effects of different formulation/process variables; (ii) phase fractions, (iii) surfactant concentration and its affinity (HLB), (iv) stirring speed, and (v) temperature on encapsulation efficiency and (multiple) drop size in freshly-prepared multiple emulsions, in order to outline an optimum formulation, and finally (vi) to modify Brugmann's law of single emulsions to relate primary and secondary emulsions phase fractions and salt content to conductivity of a multipel emulsion, and so pedict the encapsulation efficiency from conductivity measurements.

Methodology: W/O/W multiple emulsions are prepared using two-step method. Paraffin, distilled water, and two surfactants (Span 80 with HLB=4.3 to stabilise the primary emulsion, and Tween 80 with HLB=15 to stabilise the secondary emulsion) are used as a model system.

Conductivity variations are monitored during emulsification, drop size is measured using Mastersizer. Microscopic images of the final emulsions are acquired using optical microscopy.

- **Synthesis of hydrogel particles**

The aim of this minor part of the research is to introduce a modification to the common emulsification technique to produce alginate hydrogel particles. The new method is based on addition of a calcium chloride W/O emulsion to a sodium alginate W/O emulsion. Crosslinking reactions occur in sodium alginate drops while calcium chloride diffuses through the gel film formed at the interface of drops.

The objectives are to study (i) the possibility of inclusion of oil into drops of sodium alginate, (ii) synthesis of alginate hydrogel particles via mixing W/O emulsions of sodium alginate and calcium chloride, (ii) the effect of concentrations of sodium alginate and calcium chloride on the size and morphology of the synthesised hydrogel particles.

Methodology: W/O emulsions of sodium alginate and calcium chloride solutions are prepared and mixed for a period of time and formation of hydrogel particles are observed. Size and morphology of wet and dried hydrogel particles are studied using optical microscopy and scanning electron microscopy (SEM), respectively.

THESIS STRUCTURE

ABSTRACT

A summary of this work's aim and objectives, methodology and the key results are mentioned.

INTRODUCTION

The topics investigated in this work, the motivation behind them, and the aim and objectives of this research are introduced.

CHAPTER ONE LITERATURE REVIEW

In the first section of this chapter a comprehensive review of related literature in the field of catastrophic phase inversion emulsification, for instance, a general view of the possible parameters that affect catastrophic phase inversion to produce fine emulsions is presented.

In the second section of this chapter, a detailed review of related literature in the field of multiple emulsions, such as the possible parameters that affect the encapsulation to produce stable multiple emulsions, is presented.

In the third section of this chapter, a review on hydrogel particles features and methods of synthesis, and factors affecting their morphology are presented.

CHAPTER TWO MATERIALS AND METHODOLOGY

A detailed description of the materials and the experimental set-up used in this research are presented. The employed preparation methods for single emulsions, multiple emulsions and hydrogel particles are stated, and the experimental characterization methods are also explained.

CHAPTER THREE RESULTS AND DISCUSSION

All experimental results are presented and discussed in this chapter. For catastrophic phase inversion study, the mechanism of phase inversion (inclusion), and the effects of different parameters such as phase fraction, surfactant affinity/concentration/ location, temperature, and stirring speed on inversion time and drop size are disucssed based on the experimental results. For multiple emuslions study, first the possible mechanism of release is discussed, and then the effects of process and formulation variable such as stirring speed, surfactant concentration, phase fractions and oil viscosity on

encapsulation efficiency of freshly-prepared multiple emulsions are analysed and discussed.

Finally, the results of a preliminary study on a new emulsification method for synthesising alginate hydrogel particles are presented, and the effects of different variables on the size and morphology of the particles are discussed.

CHAPTER FOUR CONCLUSIONS AND RECOMMENDATIONS

Detailed conclusions from each part of the research are presented, following by recommendations for future work.

NOMENCLATURE

An alphabetic list of parameters, abbreviations, and their definitions are given.

REFERENCES

The bibliographies of all the materials used in this research are outlined.

APPENDICES

Appendix A: Experimental reproducibility.

Appendix B: Optimum salt concentration in multiple emulsions.

Appendix C: Research outcomes (list of journal manuscripts and conference publications).

Appendix D: Risk assessments forms.

Appendix E: Certificates of training sessions participated to develop the postgraduate key skills.

Chapter 1

LITERATURE REVIEW

This chapter presents a literature review that defines the main notions used throughout this work and summaries the scientific knowledge on phase inversion emulsification, multiple emulsions and hydrogel particles, with a focus on their preparation and characterisation.

1.1. Phase Inversion Emulsification

1.1.1. Introduction

This section reviews the published literature regarding the experimental studies carried out on phase inversion emulsification, in particular catastrophic phase inversion. The literature survey is focused on the experimental techniques used in the investigation of formation of fine emulsion and mechanism of phase inversion. The literature review concentrates on the factors affecting catastrophic phase inversion relevant to the materials used in the work of this thesis.

Emulsions

An emulsion refers to the dispersion of droplets of one liquid in another one with which it is incompletely miscible (Israelachvili, 1994). These liquids are usually oil and water, with one liquid dispersed as drops in a second liquid, which forms a continuous phase. Emulsions are usually prepared using high-pressure homogenisers, ultrasound, rotor/stator systems such as, mechanically stirred vessels or colloid mills. Emulsions are thermodynamically unstable and tend to separate into two phases as soon as stirring ceases. Phase separation occurs because droplets generally merge with each other when they collide. The driving force for the phase separation process is the fact that the contact between oil and water molecules is thermodynamically unfavourable because of the hydrophobic effect (McClements and Weiss, 2005).

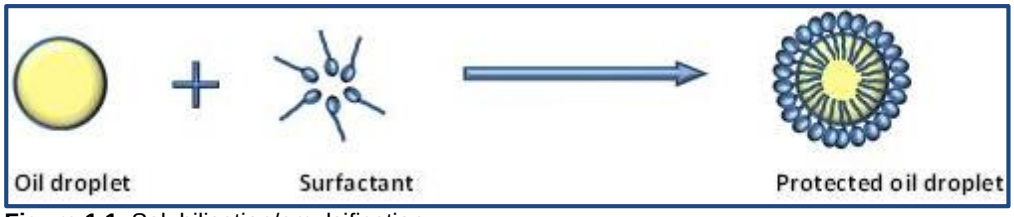


Figure 1.1. Solubilisation/emulsification.

To make a stable emulsion (fine emulsion), a third component, a surfactant, must be present in order to protect the newly formed droplets against immediate re-coalescence, see Figure 1.1. The preparation of emulsions involves many degrees of freedoms, including the emulsification process and conditions, the type and amount of surfactants, the location of surfactants and the presence of electrolytes and cosurfactants, etc.

Emulsion types

All emulsions are recognised as a mixture of two or more immiscible liquids. There are two types of emulsions: single emulsions and multiple emulsions. Depending on which type of phase forms the continuous phase, single emulsions can be classified into two different types of single emulsions: water in- oil (W/O) and oil-in-water (O/W), see Figure 1.2.

Oil-in-water (O/W): Oil droplets are dispersed in a continuous aqueous phase. This emulsion is generally formed when a hydrophilic emulsifier is used. These are used for oral administration and cosmetics and they are useful as water washable drug bases.

Water-in-oil (W/O): Aqueous droplets are dispersed in a continuous oily phase. This emulsion is generally formed when a hydrophobic emulsifier is used. These are used for cosmetics and are employed for the treatment of dry skin and emollient applications. The type of emulsion used (W/O or O/W) is affected by many factors, such as the volume of the two phases, the nature and concentration of the emulsifying agent, the temperature and the presence of additives.

There are also another two categories of multiple (double) emulsions**: water-in-oil-in-water (W/O/W) and oil-in-water-in-oil (O/W/O)**, see Figure 1.2. W/O/W emulsions are water droplets dispersed in oil droplets that are in turn dispersed in a continuous water phase. Compared to single emulsions, double emulsion droplets can be quite large (tens of mm) and they can contain many droplets from the final internal phase.

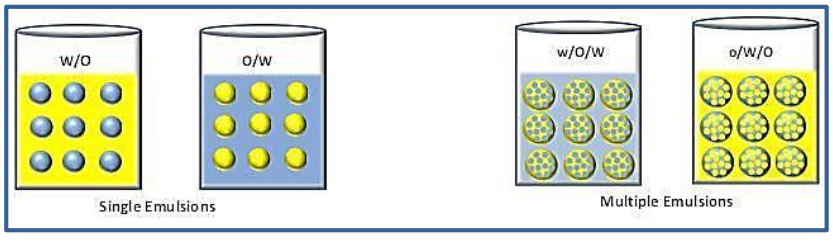


Figure 1.2. Classification of emulsions.

Surfactants/surface-active agents

Surface-active agents (surfactants) are considered to be important chemicals that are widely used in industrial processes. Due to their dual nature, including their solubility and interfacial activity, they are linked with many useful interfacial phenomena (Binks, 1998). As soon as they are added to a mixture of water and

oil they start to accumulate and be adsorbed at the interface as a thin layer or film surrounding the droplets from the continuous phase. This film prevents the droplets from contact and coalescing, which would cause changes in the interfacial composition. Using a higher amount of the surfactant (to a certain level) is favourable for the preparation of stable emulsions, providing constancy in their properties with time (Derkach, 2009). Surfactants are organic molecules that belong to a large class of molecules called amphiphiles. A single molecule usually consists of two parts hydrophilic head group, which is water soluble, and a hydrophobic tail group, which is water insoluble. For this reason, surfactant molecules have the ability to orient at interfaces, thereby altering the physical properties of the interfaces. The properties of surfactant molecules are ruled by the properties of their head and tail sections.

Surfactants Types

Surfactants can be classified into four different groups according to the charge in their head group as follow:

Anionic surfactants: usually have a negative charge on their head group, for example, sulphate groups ($-SO_3^-$). Due to their high level of toxicity, they are rarely used in the preparation of an actual food. This type of surfactant is used in many industrial applications due to its low cost and easy manufacture. They can be used as detergents and they are also very effective in stabilising emulsions. ***Cationic surfactants*** have a positive charge on their head group, for example, the trimethylammonium ion ($- N(CH_3)_3^+$) –. Due to their unusual properties, they are very useful for some specific applications. They are found in asphaltic emulsions and coatings, inks and wood pulp dispersions. Many of them are bactericides used to sterilise food bottles or containers, particularly in the dairy and beverage industries (Salager, 2002). These surfactants are more expensive than anionic surfactants due to the high-pressure hydrogenation reaction that needs to be undertaken during their manufacturing. ***Non-ionic surfactants*** have a strong affinity for water as they have a highly polar (non-charged) moiety, such as polyoxyethylene ($-OCH_2CH_2O-$) or polyol groups. They do not produce ions in the water phase. Therefore, they are well-suited to other types of surfactants and are excellent candidates to enter complex mixtures, as found in many commercial products (Salager, 2002). They can be used with high salinity or hard water due to their low sensitivity to electrolytes. Non-ionic surfactants are found today in

many industrial products, such as in pharmaceuticals, cosmetics and food products. Their importance over ionic surfactants is attributed to the length of their hydrophilic as well as hydrophobic groups, which can be varied to increase their efficiency. They are more environmentally friendly because they are easily biodegradable (McClements and Weiss, 2005). ***Zwitterionic (or amphoteric) surfactants:*** combine both a positive and a negative group. These types of surfactants are function well in high electrolyte formulations and are compatible with all other classes of surfactants. They are well suited for use in personal care and household cleaning products because they have excellent dermatological properties.

Role of surfactants in emulsion formation

When two immiscible liquids such as oil and water are mixed together, a small film will form around both liquids; this film is called the interface film. Thus, it is possible to define the interfacial area (the interface of two immiscible liquids) as the area (film) that separates the oil from the water. Interfacial tension (σ) in turn can be defined as resistance forces that prevent the two liquids from breaking up into smaller particles. Interfacial tension is considered to be one of a number of very important characteristics that should be taken into consideration in emulsion formation as it directly affects the physicochemical properties of the emulsions (Everett, 1988). There are many parameters affecting interfacial tension: the temperature and the presence of salt, surfactant, etc. (Gaonkar, 1992). Surfactants or emulsifiers help in lowering the interfacial tension. When surfactants are added to a mixture of water and oil, and because of their surface activity, they will accumulate and be adsorbed on the surface as a thin layer or film surrounding the droplets of the dispersed phase. This film will prevent the droplets from contact and coalescence, which would cause changes in the interfacial composition. This satisfies the amphiphilic nature of the surfactant, with the hydrophobic tail groups being situated in the oil phase and the hydrophilic head groups in the aqueous phase. In emulsions, surfactants perform two different actions. The first action is that of promoting the formation of an emulsion, making the emulsion easier to prepare, and aiding the stability of emulsions by any or all of the following: producing a finer particle size, reducing the interfacial tension between the oil and water and absorbing at the liquid-liquid interface (Davis, 1994; Jahanzad et al., 2009). For maximum efficiency and

effectiveness, good surfactants have limited solubility in both the oil and the water phase of the system. Emulsions also tend to be more stable when there is tighter packing of hydrophobic groups at the oil/water interface (Schramm, 2005). The second action of the surfactant, which occurs along with the preparation of the emulsion, consists of controlling the type of emulsion to be formed (O/W or W/O). According to Bancroft's rule, a surfactant has to be dissolved in the suitable continuous phase (where it has to be soluble) (Bancroft, 1913).

Hydrophilic-lipophilic balance (HLB) of the surfactant

Griffin (1949) introduced the concept of hydrophilic-lipophilic balance (HLB). This provides an indication of the relative strength of the hydrophilic and hydrophobic portions of the surfactant molecule, which can be used to classify non-ionic surfactants and to characterise their relative affinity to forming O/W and W/O emulsions (see Figure 1.3).

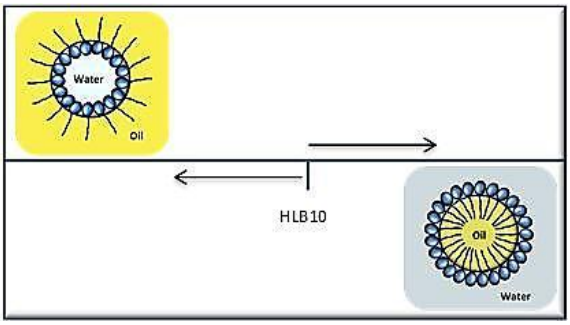


Figure 1.3. HLB scheme.

HLB of the surfactant can be calculated using the following formula:

$$HLB = \frac{1}{5}\left(\frac{M_H}{M_T} \times 100\right) \quad (1.1)$$

M_H is the molecular mass of the hydrophilic part of the surfactant molecule. M_T is the molecular mass of the entire molecule, providing a resulting scale from 1 to 20. A high HLB number generally indicates good surfactant solubility in water, while a low HLB number indicates a high relative affinity towards the oil phase. As a result, surfactants with a high HLB tend to stabilise oil-in-water (O/W) emulsions, whilst surfactants with low HLB tend to stabilise W/O emulsions. Generally, an HLB number within 10–16 is appropriate for the preparation of O/W emulsions, depending on the oil properties. This provides a large degree of freedom to select a proper pair of surfactants with a different individual HLB;

however, the HLB should not be significantly different because the stability of the emulsion is reduced when a wide HLB difference exists. HLB numbers smaller than 8 usually promote W/O emulsions. At an intermediate HLB (8–11), the surfactant has a balance in favour of the oil and water phase (see Table 1.1). As a result, any large variation in the average HLB of an emulsion may lead to a transitional phase inversion (TPI) to the opposite emulsion (Sajjadi, 2006a). The HLB is an indicator of the surfactant characteristics of an emulsifier, but not its efficiency. Thus, all emulsifiers with a high HLB will tend to promote O/W emulsions; there will be a considerable variation in the efficiency with which those emulsifiers act in any given system (Schramm, 2005). For instance, mixtures of surfactants typically produce emulsions that are more stable than single surfactants of the same HLB.

Table 1.1. Application of surfactants depending on their HLB.

Application	HLB
1-3	Antifoaming agents
3-8	W/O emulsifiers
7-9	Wetting agents
10-16	O/W emulsifiers
13-16	Detergents
15-16	Solubilises

Davies (1957) suggested a different competitive coalescence kinetic model to describe the type of emulsions and a new scale to calculate the HLB values:

$$HLB = \sum H_{h,i} - \sum H_{l,i} + 7 \qquad (1.2)$$

$H_{h,i}$ and $H_{l,i}$ are the contributions of the hydrophilic and hydrophobic parts respectively, where the effects of stronger and weaker hydrophilic groups are differentiated. However, both Griffin and Davies' scales are not defined for all categories of polar heads and there is, for instance, no possibility of classifying phospholipids, fluorinated or sucrose ester surfactants, and the correlation between HLB values from Davies and Griffin is not always clear. It is difficult or impossible to use these two (Griffin and Davies' scales) methods in many food and cosmetic surfactant mixture applications.

Cloud point (CP)

Clouding performance, is defined as a physical change in the homogeneous solutions of amphiphilic substances, due to which the solution separates into two phases, the surfactant-rich and the surfactant-poor phase, at a specific

temperature. This temperature is known as the cloud point (CP) (Chai and Mu, 2002).

Generally, most surfactants have aconsiderable solubility in water. Any changes in the solution temperature will lead to changes in hydrophobic tail length and head group nature, which will affect the solubility of the surfactant.

Non-ionic surfactants have an oil-soluble group attached to a water soluble polyoxyethylene chain. By increasing the temperature of their aqueous solution to a certain value, cloud point, much larger particles will be formed and non-ionic surfactants will show clouding performance (becoming visibly turbid) (Chai and Mu, 2002; Bordier, 1981). Due to the sensitivity of the hydrogen bonding between the solvent and the ether oxygen atoms in the polyoxyethylene chain to the temperature, at which the cloud point dehydration for this bond occurs, the surfactant is no longer water soluble and it starts to separate from the water (Mukherjee et al., 2011). Increasing the temperature further leads to phase separation, which is reversible (Alauddin et al., 2009). Generally, non-ionic surfactants show an optimal effectiveness when used near or below their CP and, hence, CP of surfactant solutions can be strongly influenced by the presence of other materials (Mukherjee et al., 2011; Sharma et al., 2003).

1.1.2. Emulsification processes

Emulsification is generally carried out by one of two different techniques: the direct (D) or the phase inversion (PI) method (see Figure 1.4).

Direct method (D)

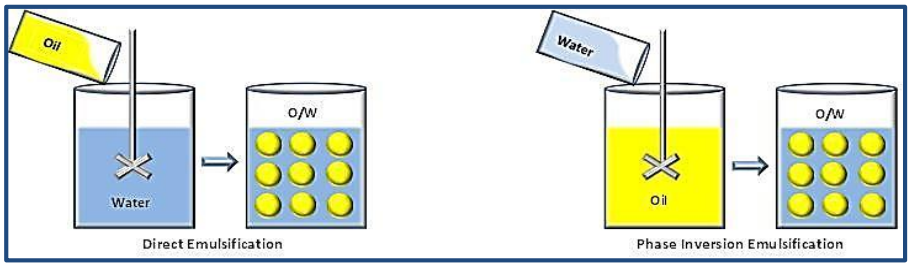


Figure 1.4. Direct and phase inversion emulsification (preparation of O/W emulsions).

In this technique, the dispersed phase is simply added to the continuous phase under an intensive stirring speed (Jahanzad et al., 2009). This is called direct emulsification because the type of initial emulsion is that of the desired emulsion.

Phase inversion emulsification

Phase inversion is an attractive method by which to prepare emulsions as it allows for the formation of a finer drop than the conventional emulsification process, and it uses a lower energy input (Perazzo et al., 2015). In this method, the phase that is intended to be the continuous phase is added to the intended dispersed phase until phase inversion occurs, from oil-in-water (O/W) to water-in-oil (W/O) or vice versa, until the desired emulsion type is formed (Groeneweg et al., 1998; Hu et al., 2005; Yeo et al., 2002; Kumar, 1996). Therefore, this is a process by which a water-in-oil (W/O) emulsion transforms into an oil-in-water (O/W) emulsion or vice versa. The most common method by which to detect phase inversion is to observe emulsion conductivity. Phase inversion takes place when there is a significant change in the conductivity. It is well known that the degree of some of the emulsion properties, such as stability and viscosity changes suddenly when phase inversion takes place. Continuous monitoring of both emulsion conductivity and viscosity assists in identifying several phenomena that might occur during the inversion process. This might include droplet size variations near phase inversion and information about intermediate morphologies exhibited around phase inversion (Lv et al., 2014). Phase inversion in emulsions was first reported by Reynolds (1921) when he stated that phase inversion occurred after a change of temperature. Depending on the changed variable, one of two different methods is used to prepare emulsions through the phase inversion process: transitional phase inversion and catastrophic phase inversion (CPI) (Brooks and Richmond, 1991; Vaessen and Stein, 1995; Sajjadi et al., 2002b; Rondón-González et al., 2006a; Campbell et al., 2012; Perazzo et al., 2015). Phase inversion is induced by many factors, such as surfactant affinity and dynamic factors.

Catastrophic phase inversion (CPI)

Catastrophic phase inversion is believed to be caused by increasing the volume fraction of the dispersed phase to the continuous phase until inversion occurs in a sudden manner (Lv et al., 2014). It usually starts with abnormal emulsions: emulsions in which the surfactant has more affinity toward the dispersed phase (Jahanzad et al., 2009). Abnormal emulsions do not obey Bancroft's rule and are extremely unstable. Usually, vigorous stirring is required to maintain the dynamic stability of abnormal emulsions. During the catastrophic phase inversion process,

the instability of an abnormal emulsion continuously increases until it inverts to a normal emulsion of the opposite morphology.

CPI is triggered by increasing the drop collision rate and, hence, increasing rate of coalescence. As soon as the dispersed phase volume reaches a threshold such that the drops are in contact with one another, coalescence increases rapidly; at a certain point, the continuous phase becomes submerged by the coalescing drops and the emulsion is inverted to become the opposite morphology (Liu et al., 2012). This can be caused by the addition of a surfactant, salt or another ingredient, the viscosity of the phases, continuous stirring of the emulsion and altering temperature whilst adding to the dispersed phase (Rondón-González et al., 2006a). As a result, this type of phase inversion is not necessarily related to the presence of surfactants. However, in the presence of a surfactant, CPI is capable of producing fine drops. It was reported that CPI can be accompanied by the formation of multiple drops prior to inversion (Pacek et al., 1994; Sajjadi et al., 2000, 2002a, 2003a). For instance, an initial W/O emulsion will form an O/W/O emulsion before inverting to a final O/W emulsion. It is also reported that catastrophic phase inversion usually produces finer emulsions than the direct emulsification method (Thakur et al., 2008; Sajjadi, 2006a).

Transitional phase inversion (TPI)

This is also known as low-energy emulsification. Transitional is a term introduced by Salager to describe the emulsion inversion reached by changing the water/oil fraction (Salager et al., 1979). In this technique, phase inversion results from changing the formulation variable, such as the surfactant affinity toward the two phases; this is done by changing the temperature, changing the composition of the surfactant mixture at a constant temperature, and the addition of salt or other additives (Vaessen and Stein, 1995; Xie and Brooks, 2005; Jahanzad et al., 2009; Perazzo et al., 2015). Different types of surfactants can be used to enhance transitional phase inversion, such as a combination of anionic and non-ionic surfactants, non-ionic surfactants, a combination of anionic surfactants and co-surfactants, or inorganic stabiliser particles with different HLB (Sajjadi et al., 2004). In order to attain a transitional inversion, the surfactant concentration must exceed the CMC value (Sajjadi et al., 2003b). The affinity of a surfactant toward a phase is conventionally measured by its hydrophilic-lipophilic balance (HLB). The

HLB of a surfactant measures the degree to which it is hydrophilic or lipophilic. Altering the HLB results in the surfactant being attracted more towards the oil or water phase, depending on the direction of change.

Phase map

The surfactant affinity and the water-to-oil fraction required to prepare an abnormal emulsion and to apply an inversion protocol are easily identified in a formulation-composition bidimensional map (Rondón-González et al., 2006a), see Figure 1.5. The map is divided into three vertical regions. Region A represents a mid-range of water-to-oil fraction, while the B and C regions are represented with low and high water content respectively. Multiple emulsions of the O/W/O and W/O/W type are suddenly formed upon stirring in the B− and C+ regions respectively. single W/O (respectively O/W) emulsions are produced in the A+ and B+ (respectively A− and C−) zones. The positive sign after the letter represents that the surfactant affinity toward the oil phase dominated, while the negative sign represents the opposite case. HLD = 0 represents the optimal formulation at which surfactant affinity toward the oil phase is equal to that of the water phase. At this formulation, many systems exhibit three-phase behaviour, with a microemulsion middle phase in equilibrium with excess oil and water (Rondón-González et al., 2006a).

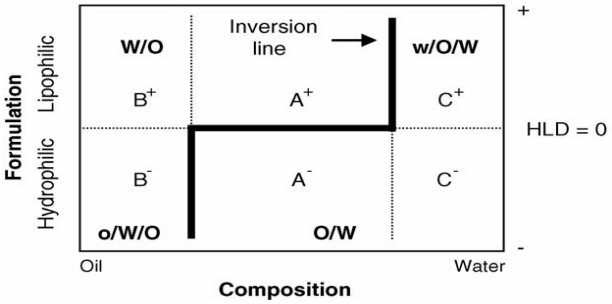


Figure 1.5. Bidimensional formulation-composition map illustrating emulsion types and standard inversion frontier (Rondón-González et al., 2006a).

Optimal formulation (HLD = 0) coincides with the horizontal branch of the standard inversion line (stair-like bold line in Figure 1.5), which is determined by stirring an equilibrated surfactant-oil-water system and measuring its electrolytic conductivity. The trespassing of the horizontal branch produces a transitional inversion while crossing over the lateral branches corresponds to a catastrophic inversion. The central (horizontal) branch of the inversion line is always in the same location (optimal formulation), whereas the lateral (vertical) inversion

branches can move toward the centre or the sides of the map depending on the protocol and other variables such as phase viscosity or surfactant concentration. As a result, all effects can be rendered in a three-dimensional diagram, as introduced by Salager et al. (2000). Rondón-González et al. (2006a) introduced the influence of the water-to-oil fraction and the phase behaviour in the emulsion inversion produced by continuous stirring only. He interpreted phase inversion in a two-dimensional formulation-composition map. Since the formulation and composition remain constant, the point that represents the system in Figure 1.5 map does not change position. Hence, the emulsion inversion is due to a change in condition, which shifts the A+/C+ branch of the inversion line from left to right as the stirring goes on.

Emulsion stability

Emulsions are mixtures of two immiscible liquids, usually oil and water, in which one liquid is dispersed as droplets (dispersed phase) into the other (continuous phase) with the aid of mechanical energy. Emulsion stability is a term used to describe the process by which emulsions are made to resist changes in their properties over time; the more stable the emulsion, the more slowly its properties change. Due to oil/water immiscibility, and if the energy stops, emulsions will tend to phase separate at thermodynamic equilibrium (Perazzo et al., 2015), forming two layers to minimise the contact area between the two phases. The liquid with the smaller density, the oil phase, will move upward into the system, while the one with higher density will move downward into the system. This process is called phase separation. Therefore, emulsion stability is one of the main problems in various industrial applications (Perazzo et al., 2015).

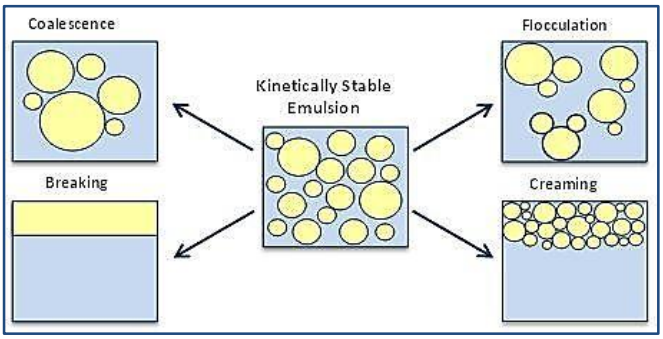


Figure 1.6. Emulsion instability.

There are four main phenomena driving phase separation that are put into motion when this process is in place: breaking, creaming, sedimentation flocculation and

Brownian flocculation (see Figure 1.6). Flocculation and creaming are the primary methods by which the emulsions are destabilised; however, they can all occur simultaneously.

Creaming, flocculation and coalescence have all been found to occur when an emulsion of limited stability is centrifuged at low speed or for various amounts of time (Binks, 1998). Creaming occurs as a result of the movement of oil drops in a centrifuge, causing a concentrated layer to form at the top of the sample; this happens as a result of the density difference between droplets and surrounding liquid, causing, in effect, the appearance of a boundary between the upper cream layer and the lower emulsion layer.

Creaming is a form of gravitational separation and it occurs as a result of the upward movement of low-density droplets in the liquid. Sedimentation, on the other hand, although another form of gravitational separation, describes the downward movement of higher density droplets against the surrounding liquid. Coalescence and flocculation are both types of droplet aggregation, and, like creaming and sedimentation, whilst these processes are similar, different processes are taking place here: flocculation is a result of two or more droplets forming an aggregate but retaining their individual forms; in coalescence, however, these droplets merge to form a single larger droplet. Excessive coalescence can lead to a separate layer of oil forming at the top of the sample.

In order to prepare emulsions that are kinetically stable, surfactants must be added to lower interfacial tension between the two phases. Emulsions with fine drops are more stable against phase separation and widely exploited in application, which is also due to their capacity to incorporate hydrophilic or hydrophobic species.

1.1.3. Applications of emulsions

Emulsions are very important and widely used in our everyday lives and multiple examples are available of their various applications; they are widely used in many major chemical and oil industries (Chappat, 1994). In the pharmaceutical industry, they are used to make medicines with a more appealing flavour and to improve value by controlling the amount of active ingredients. Emulsions are frequently used in pharmaceutics, hairstyling, personal hygiene and cosmetics. These are usually oil and water emulsions, but which is dispersed and which is continuous depend on the formulation. These emulsions may be called creams,

ointments, pastes, films, or even liquids depending on their oil and water proportions and their method of administration. Microemulsions are used to deliver vaccines and kill microbes. Some types of nanoemulsions have also been shown to effectively destroy HIV and various tuberculosis pathogens. The most effective application of nanoemulsions for hygiene is for the disinfection of surfaces. Typical nanoemulsions include soybean oil, with drops that are 400-600nm in diameter. In food, oil-in-water emulsions are common as the cream in espresso (coffee oil-in-water), Mayonnaise (vegetable oil in lemon juice or vinegar, with egg yolk lecithin as the emulsifier), etc.

In the oil industry, oil-in-water emulsions are important as a displacing fluid for enhanced oil recovery. Oil-in-water emulsions in the presence of surface-active agents or emulsifiers significantly reduce the interfacial tension between trapped oil and displacing fluid and they stabilise the interface against coalescence once it is formed. Emulsions with dispersed phase drops effectively block the more permeable paths and force the displacing fluid to flow through the unswept regions, which increases the overall sweep and displacement efficiency, leading to an increase in oil recovery**.** Various microemulsion systems are used in different areas of the oil industry; for example, in well hydraulic fracturing, as corrosion inhibitors in pipelines, in the breakdown of water/oil emulsions, in enhanced oil recovery, and as an alternative fuel. When a microemulsion is used as a fracturing fluid, it has properties that are compatible with those of polymeric fluids, with the advantage of reducing the formation of damage. As corrosion inhibitors in pipelines, microemulsions interact with the metallic surface, although less intensively than surfactants in solution. Microemulsions have shown good anticorrosive potential. In the breakdown of water-in-oil emulsions, microemulsions have presented very good de-emulsifying characteristics. In enhanced oil recovery, microemulsion systems have proved to be efficient in the oil extraction process. Microemulsified systems containing neat diesel or a mixture of diesel with different percentages of vegetable oils can be used as alternative fuels, showing improved combustion properties in diesel engines (Binks, 1998).

1.1.4. Catastrophic Phase Inversion (CPI)

Based on the thermodynamic point of view only, some authors have described catastrophic phase inversion as a “catastrophic event” because it appears to be a

rapid change in morphology caused by a gradual change in experimental conditions (Bouchama et al., 2003). The term “catastrophic” was chosen by Salager (1982) after Dickinson (1981) suggested that the catastrophe theory may be applicable to describe phase inversion in emulsions. This suggestion was based on the fact that emulsion phase inversion, induced by increasing the volume fraction of the dispersed phase, displays some qualitative characteristics of the so-called cusp catastrophe theory developed by Thom (1974). Despite the fact that this approach has been successfully applied to describe the qualitative features of catastrophic phase inversion (CPI) in emulsions, it has failed to be considered as a predictive model (Vaessen and Stein, 1995). Conversely, catastrophic phase inversion has been treated on a kinetic basis rather than a thermodynamic basis. Some researchers have suggested that CPI should be modelled on the basis of coalescence kinetics (Gilchrist et al., 1989; Vaessen and Stein, 1995; Kumar, 1996).

To explain the phase inversion mechanism, Pacek et al. (1994) suggested that the development of a multiple emulsion structures is related to phase inversion. Similarly, other researchers have shown that the catastrophic phase inversion process is associated with the formation of multiple emulsions by inclusion wherein the external phase is continuously included as drops inside the original dispersed phase drops (drop inside drop), which results in a growth of the effective dispersed phase volume until a critical value at which the inversion occurs (Jahanzad et al., 2009; Tyrode et al., 2003; Sajjadi et al., 2002b; Pacek, 1994; Brooks and Richmond, 1994a, 1994b, 1994c, 1994d; Groeneweg, 1998). Ostwald indicated that phase inversion takes place when the system reaches a critical close packing condition (Ostwald, 1911), while Brooks and Richmond claimed that if a multiple emulsion is formed, the effective volume fraction of the dispersed phase is much higher than the actual volume fraction of the dispersed phase component. Consequently, phase inversion should take place at a lower volume fraction than in the absence of enclosed droplets of the continuous phase (Brooks and Richmond, 1991, 1993).

1.1.4.1. Factors Controlling CPI Process

The phase inversion process can be controlled by two different factors: surfactant affinity toward phases and dynamic effects (Binks, 1998). Surfactant affinity can also be controlled by a number of factors, such as:

- Oil type
- Surfactant type and concentration
- Temperature of the system
- Water phase fraction
- Additives in the oil or water phase
- While the dynamic effects can be controlled by:
- Agitation intensity
- Rate and order of addition of the different components.

Massive research works have been carried out on catastrophic phase inversion to investigate the effect of different formulations that lead to producingfine emulsion of a desired stability, below is a review of some of these works.

Effect of temperature

The effect of temperature on phase inversion in emulsions was first reported by Reynolds (1920). In 1964 Shinoda and Arai were the first to point out the effect of temperature on phase inversion (Shinoda and Arai, 1964) using non-ionic surfactants. They reported the relationship of the phase inversion temperature and the cloud point of such surfactants. Shinoda et al. (1971) further described the association between phase inversion temperature and HLB of the surfactant. Later, phase inversion temperature (PIT) was suggested by Kunieda and Shinoda (1985) as a method to classify non-ionic surfactants. They found that after increasing the temperature, non-ionic surfactants become more hydrophobic so surfactants change their chemical configuration and physical arrangement, thereby encouraging phase inversion to occur. Compared to non-ionic surfactants, the effect of temperature on the HLB of ionic surfactants is relatively small. With non-ionic surfactants, the hydration forces between the hydrophilic moiety of the surfactant and water are stronger at lower temperatures, thus making the surfactant more hydrophilic. Therefore, with a temperature increase, an O/W emulsion might invert into a W/O emulsion (Sajjadi et al., 2004). PIT is defined as the temperature at which the affinity of non-ionic surfactant for both phases is equal (Vaessen et al., 1996).

Effect of the HLB of the surfactant

The emulsification process is usually characterised by the degree to which it allows the surfactants, as well as the dispersed phase, to be integrated into

emulsions. There are different types of surfactants that can be used for making emulsions, but for all emulsion systems, choosing the right surfactant are of crucial importance; a single surfactant also cannot produce the desired stability, therefore a surfactant with a broad chain length distribution must be used. Non-ionic surfactants offer certain advantages over conventional ionic emulsifiers, particularly in their ability to make systematic changes in the hydrophilicity of the surfactant (Sajjadi et al., 2003a). Applications of a mixture of surfactants with different hydrophilicity, because of their lower interfacial tension or their higher capacity for solubilisation, are usually used to enhance the emulsion stability (Becher, 2001; Sajjadi, 2006a). Lower interfacial tension means less resistance force against drop break-up and a finer emulsion. The affinity of a surfactant toward a phase is measured by its hydrophilic-lipophilic balance (HLB) (Sajjadi et al., 2004). If the surfactant composition, the affinity of the surfactant, in an emulsion is changed from hydrophilic to hydrophobic or vice versa, phase inversion to the opposite morphology can take place (Marszall, 1975).

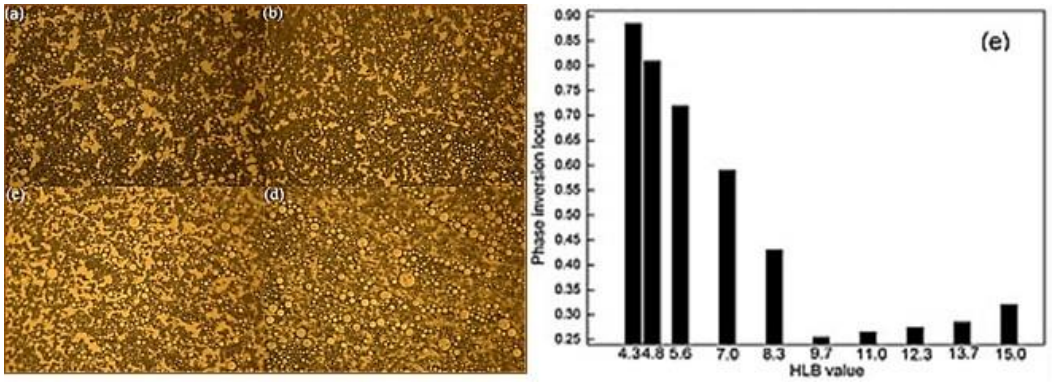


Figure 1.7. Micrographs of water/Span 80 (2 wt.%)-Tween 80 (2 wt.%)/oil emulsions with water volume fraction of 20% (a), 25% (b), 30% (c) and 40%(d). Bar equal to 25 _m, (e) Phase inversion locus against HLB values, (Lv et al., 2014).

Many researchers have explored the effect of HLB of the surfactant on CPI (Davis, 1994; Boyd and Sherman, 1972; Jahanzad et al., 2009; Sajjadi et al., 2003a; Rondón-González et al., 2009). Lv et al. (2014) studied the preparation of emulsions using catastrophic phase inversion and various surfactants. They observed the changes in the emulsion conductivities during phase inversions. They also discussed the influence of HLB values on phase inversion locus using mixed surfactants (2 wt.% Span 80 and 2 wt.% Tween 80), see Figure 1.7.

The average diameter of the droplets of both W/O and O/W emulsions decreased when approaching phase inversion, with the minimum drop diameter therefore

existing close to the inversion point (26% water concentration). The same results were obtained using single surfactants (Span 80 or Tween 80). Figure 1.7(e) illustrates the effect of the HLB of the surfactant on phase inversion. It is clear that the correlation between the phase inversion locus and the HLB is U-shaped. When Span 80 was used as the only surfactant, phase inversion occurred until the water concentration exceeded 88% while using Tween 80, phase inversion occurred at a water concentration of 32%.

According to Bancroft's rule, Tween 80 stabilises O/W emulsions as it is a hydrophilic surfactant, while Span 80 stabilises W/O emulsions as it is a hydrophopic surfactant. This may contribute to low phase inversion locus for emulsions stabilised by Tween 80 and high water concentration for inversion as Span 80 is used separately. Furthermore, when Tween 80 was mixed with Span 80, the water volume fraction for inversion initially decreased to the minimum value at about HLB = 9.7 and then it gradually increased.

Inclusion

Sajjadi et al. (2003a) briefly reported the effect of the HLB of the surfactant on inclusion in phase inversion and how inclusion influence the final size of the dispersed droplets in the normal emulsion. In their study, they altered the HLB of the surfactant, using a mixture of hydrophobic and hydrophilic surfactants. The size of the dispersed droplets in the normal emulsion was greatly reduced as the optimum HLB was approached. They used a model abnormal O/W/O emulsion. The abnormal O/W/O emulsions were made by the gradual addition of water to the oil phase containing a mixture of a water-soluble and an oil-soluble surfactant until phase inversion to the corresponding normal O/W emulsions occurred. Their results showed that phase inversion occurred earlier, in terms of volume fraction of the water, as HLB decreased. They also reported that the inclusion of part of the continuous phase in the dispersed phase results in increasing the effective volume fraction of dispersed phase f_d and causes the inversion to occur at a lower holdup. A boost of actual volume fraction of the dispersed phase due to the formation of multiple emulsions could induce a catastrophic phase inversion over a wide range of dispersed phase volume fractions, depending on the surfactant concentration and properties. They also stated that when low HLB (large quantity of hydrophobic surfactant) was used, the stability of the dispersed water drops was enhanced and inclusion was decreased, leading to a delay in phase inversion in terms of f_w.

Similarly Rondón-González et al. (2009) reported that the global formulation of a water-oil-surfactant system, expressed as the hydrophilic-lipophilic Deviation (HLD), has a significant effect on the inversion produced by continuous stirring of an abnormal system. They reported that phase inversion could occur through the formation of a multiple emulsion (W/O/W), inclusion. In this study, two different surfactant systems were used: the first system consists of a mixture of two commercial surfactants, while the second system was formulated with only one surfactant. Either Igepal (CO-520) or Igepal (CO-210) was used separately. Igepal (CO-210) or Igepal (CO-520) was dissolved in the oil phase, while Igepal (CO-630) was added to the water. The emulsified systems before inversion were unstable because the external O/W interface was not favored by the formulation, which is globally lipophilic. As the lipophilicity of the surfactant mixture decreases and the system moves toward optimum formulation, the inversion time decreases, reaching a minimum value and then increasing as optimum formulation is approached. In all systems, phase inversion to a normal W/O emulsion occurred through the formation of a multiple emulsion (W/O/W), inclusion. In the system prepared with two surfactants, inversion time was strongly displaced to the low water fraction, while in the system prepared with only one surfactant, at high water fractions, complete inversion did not occur immediately, but only after some additional stirring time, the so-called propagation time, to complete the inversion. It was suggested that the stability of the most internal emulsion determines the kinetics of inclusion and the stirring time essential to induce phase inversion.

Effect of surfactant concentration

In a comparison between the CPI and D method, Jahanzad et al. (2009) investigated the possible conditions under which catastrophic phase inversion (CPI) can produce finer drops than the direct method (D). The drop size $D_{3,2}$ of the final emulsions obtained by CPI was compared with that obtained by the D method in terms of surfactant HLBs and surfactant concentration.

Inclusion

In their study, Jahanzad et al. (2009), a model of cyclohexane/water emulsion with a mixture of non-ionic surfactants was used. They found that applying a large amount of surfactant in the system during CPI process will lead to producing smaller drops than D process due to the formation of multiple drops,

inclusion, multiple drops forms in the pre-inversion region. Conversely, if a low amount of surfactant is applied in the system multiple drops do not form widely and the two methods produce almost the same drop sizes (see Figure 1.8). They concluded that the formation of multiple drops, inclusion, is the main reason for CPI producing finer emulsions than conventional direct emulsification (D). Multiple O/W/O drops are formed by inclusion due to the existence of the water-soluble surfactant in the water phase.

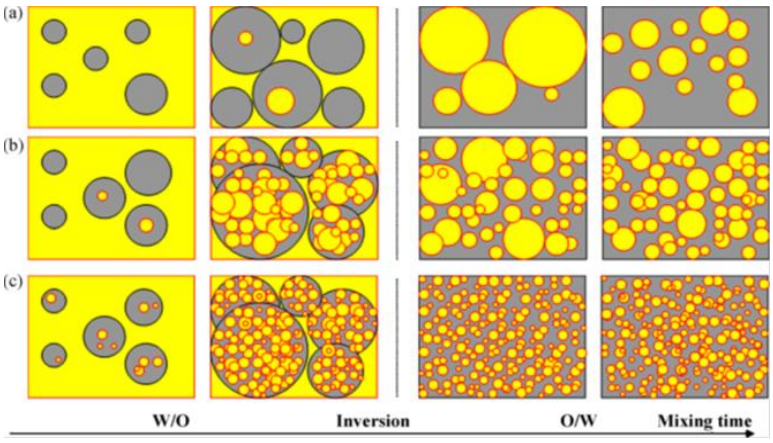


Figure 1.8. Schematic presentation of time evolution of drop size and morphology with mixing time before and after inversion; (a) low [S], (b) high [S] and high HLB and (c) high [S] and low HLB (Jahanzad et al., 2009).

Figure 1.9 displays the growth of drop size and morphology in a pre-inversion region for a typical water/PIB (W/O) abnormal emulsion during CPI. In another study, it was found that drop size decreases steeply with increasing surfactant concentration in the polar phase (oil phase) when it is within the micellar range (Liu et al., 2012).

Likewise Sajjadi et al. (2002b) concluded that catastrophic phase inversion is induced by inclusion, which continuously increases the effective volume fraction of the dispersed phase. They reported that inversion time for agitation induced CPI to be a function of the initial surfactant concentration and dispersed phase fraction. They studied catastrophic phase inversion in abnormal O/W/O emulsions using polyisobutylene as the continuous phase, water as the dispersed phase, and a water-soluble grade surfactant (dissolved in the oil phase). Although the water-soluble surfactant migrates into the interfacial area, it cannot offer enough stabilisation for the drops, and the rate of drop coalescence is high. The multiple water drops are quite unstable because they are dispersed in an oil-continuous phase containing a surfactant with low oil solubility, whilst the internal oil droplets are stable because the dispersed phase contains a water-

soluble surfactant. Therefore, the internal structure of the abnormal emulsion was strongly affected by the surfactant concentration, whilst the external phase was not affected. At a lower water volume fraction (f_w), using a higher amount of surfactant leads to an increase in the affinity of the dispersed phase to include a larger amount of oil from the continuous phase and to induce the phase inversion in the opposite morphology. In other word, inclusion was increased. The drop coalescence mechanism is responsible for inclusion at a higher surfactant loading and f_w, whilst the drop deformation mechanism is responsible for inclusion at a lower surfactant loading.

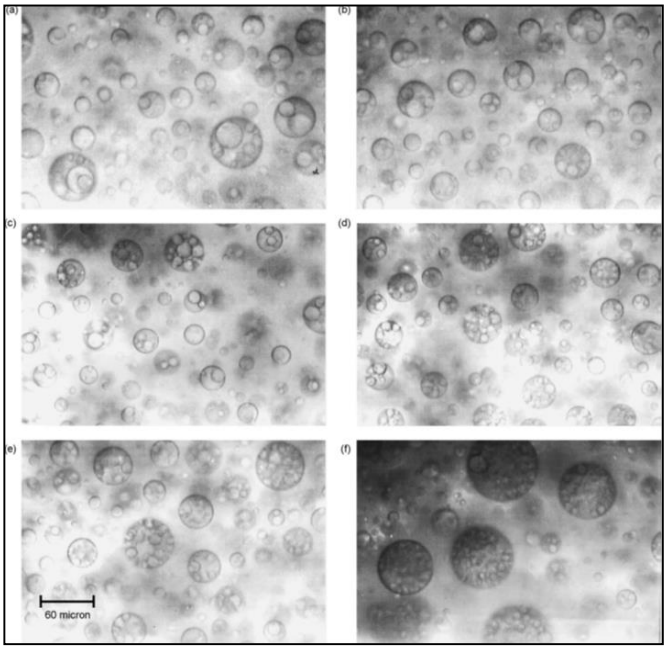


Figure 1.9. Time evolution of drop size and drop morphology for a typical water/PIB emulsion undergoing CPI; (a) 1.0 min, (b) 5.0 min, (c) 10.0 min, (d) 20 min, (e) 30 min and (f) 60min (water-in-PIB emulsion, water volume fraction = 20 vol.%, 5.0 wt.% NPE12 in both phases, stirring speed = 500 rpm, T= 60◦C. Emulsification was started batch wise by the instant addition of the water to the PIB, stirring speed = 500 rpm. Drops morphology were monitored with time) (Jahanzad et al., 2009).

Effect of the initial surfactant location

The initial surfactant location can play an important role in the dynamics of phase inversion emulsification (Lin et al., 1975; Sajjadi, 2006b). In a method called agent-in-oil (AO), the surfactant is added to the oil phase and pure water is then added to the oil/surfactant mixture to produce the final oil-in-water (O/W) emulsion. On the other hand, surfactants might be dissolved in the phase with higher affinity (e.g. lipophilic in oil and hydrophilic in water), referred to as segregated agents (SA) and water is then added to oil in order to produce an O/W emulsion.

Lin et al. (1975) studied the effect of the surfactant location on drop size and stability of emulsions; in their study, a series of emulsions were stabilised with different non-ionic surfactants and oils. Emulsions were prepared by initially placing the surfactant into the oil phase to produce finer and more stable emulsions than if the same formulation had been made by initially placing the surfactant into the water phase. In a system prepared by initially placing a hydrophilic surfactant in the oil phase, a portion of the water phase was added during the emulsification process to form a W/O emulsion. This emulsion eventually inverted to form an O/W emulsion as the surfactant migrated to the water phase. Multiple emulsions of W/O/W were observed during the process. However, multiple emulsion droplets were not observed when the same surfactant was initially placed in the water phase. This could be the reason why phase inversion gives finer and more stable emulsions if the surfactant is initially placed in the oil phase prior to the emulsification process.

Effect of the dispersed phase fraction

In an experimental study, Bouchama et al. (2003) investigated the effect of the aliquots of the dispersed phase added to the mixture on the locus of phase inversion. They explained this effect on the basis of requirements for the formation of multiple emulsions. In this study, paraffin oil was used as the oil phase and water as the aqueous phase and non-ionic surfactant; Triton X-100 (polyethylene glycol 4-tert-octylphenyl ether) was dissolved in the aqueous phase. The results suggested that adding small amounts of water leads to an increase in the locus of phase inversion. When the water volume fraction is less than 0.002, the emulsion becomes trapped in its non-preferred morphology and the locus of phase inversion is shifted to a much higher volume fraction. This is due to the formation of multiple emulsions. Emulsions can only form if the droplets are large enough, or the forces acting on the droplets are strong; this correlates to the viscosity and volume fraction of the emulsion as well as the stirring speed.

Effect of mixing speed

Since emulsions are thermodynamically unstable, they will not form spontaneously and therefore energy must be provided in order to make the emulsion. This energy must be sufficient to break up one of the phases into small

droplets, which can be dispersed and stabilised in the other phase. The energy is usually provided in the form of mechanical agitation. The energy allows the oil droplets to continuously move around and collide and coalesce with neighbouring droplets.

Inclusion

Sajjadi et al. (2000) investigated the morphological variation of complex drops of oil/water/oil (O/W/O) in unstable polymer/water/surfactant. They reported for the first time that abnormal emulsions subjected to continuous agitation also undergo phase inversion. They showed that such emulsions gradually transform into multiple emulsions by the inclusion of the continuous phase into the dispersed phase until the phase inversion occurs if the required effective dispersed phase fraction is achieved. This protocol is a more convenient way to achieve CPI as the requirement for pumping the fluid is eliminated. This new protocol has been welcomed by the scientific community, which can be demonstrated by the significant number of research papers published on "agitation induced phase inversion" (Deshpande and Kumar, 2012; Jahanzad et al., 2009; Rondón-González et al., 2006a, 2006b, 2007).

There is relatively little information available on agitation induced phase inversion; however, some inferences can be drawn by comparing it with conventional CPI. Brooks and Richmond examined the changes in drop diameters and drop types that occurred during catastrophic phase inversion (to produce oil-in-water emulsions) and drop size distributions produced by inversion in the presence of a non-ionic surfactant (Brooks and Richmond, 1993). They found that in agitated liquid-liquid dispersions, the drop size distribution during catastrophic phase inversion depends on the stirring speed and the rate of addition of the aqueous phase. When non-ionic surfactants are used, the formation of O/W/O drops becomes important; the drop size before inversion is related linearly to the drop size after inversion. They also found that the size of O/W/O drops is related to the volume fraction of the dispersed phase and to the stirrer speed.

Mira et al. (2003) studied the effect of stirring speed on CPI. They found that using intermediate stirring speeds leads to a maximum delay before inversion and the process doesn't associate with the formation of multiple emulsions. However, if high or low speed is employed, the process is associated with the formation of multiple emulsions and the inversion occurs very fast.

Effect of water-to-oil fraction

Formation of multiple emulsions during phase inversion depends on many factors, such as the surfactant affinity and its concentration, viscosity of the continuous phase (Lee et al., 2002) and the rate of drop break-up and coalescence, all of which are determined by the stirring conditions (Sajjadi et al., 2002b; Salager et al., 2000). When emulsion inversion from abnormal to normal morphology is induced by continuous stirring only, the required stirring time and the kinetics of the inversion process are found to be correlated to the water-to-oil fraction (WOR) of the initial emulsion (Rondón-González et al., 2006b).

Inclusion

Rondón-González et al (2006b) showed that for intermediate initial water content ($f_w = 0.6$ and 0.7), inclusion of the water droplets inside the oil drops is found to take place throughout the entire process, whilst at the initial water content ($f_w = 0.8$), the major water inclusion takes place suddenly just before the inversion. The effect of the surfactant concentration seems to also be correlated to the initial water content. At $f_w = 0.6$, the inversion time is the same whatever the surfactant concentration, however, at a higher water content ($f_w = 0.7$ and 0.8), it seems that there is an optimal concentration (4.5 wt.%) that reduces the inversion time to its lowest value.

1.2. Multiple Emulsions

1.2.1. Introduction

Multiple emulsions are classified as a liquid dispersion in which the droplets of the dispersed phase contain even smaller dispersed droplets that are similar to the continuous phase. Multiple emulsions droplets are in most cases very polydispersed. They also defined as a structure of two liquids that separated by a third liquid (Sagalowicz and Leser, 2010; Garti and Bisperink, 1998; Gerald, 2007).

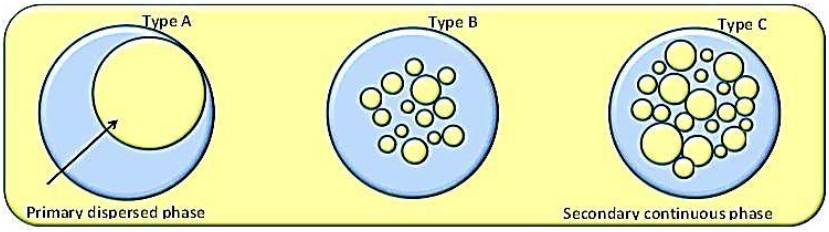


Figure 1.10. Single and multiple compartments W/O/W multiple emulsions.

Multiple emulsions are often classified into three groups depending on the number of internal droplets present in the multiple emulsion globules (Florence and Whitehill, 1981; Garti, 1997b; Pal, 2011), see Figure 1.10.

- Type A, where the multiple emulsion drops consists of only one large internal droplet, in this case, the multiple emulsion drops is of “core–shell” type.
- Type B, where the multiple emulsions drop consists of some small number of internal droplets.
- Type C, where the multiple emulsions drop consists of a vast number of internal droplets.

Multiple emulsions (types A, B or C) can be further classified as either oil-in-water-in-oil (O/W/O) multiple emulsion or water-in-oil-in-water (W/O/W) multiple emulsion (O’Regan and Mulvihill, 2010). ***Water-in-Oil-in-Water (W/O/W) emulsions*:** they are systems in which the primary emulsion, (W/O), is dispersed in a secondary aqueous phase. In other words, W/O/W emulsions are systems in which oil droplets are enclosed by an aqueous phase, which in turn these oil droplets surrounds one or more water droplets (Vladisavljević, 2016). Water-in-oil-in-water (W/O/W) multiple emulsions are the most commonly studied multiple emulsions because most food emulsions have an aqueous continuous phase, and there is a greater availability/selection of food grade hydrophilic emulsifiers

that can be used to stabilize the dispersed oil phase (Pays et al., 2002; O'Regan and Mulvihill, 2010,). However, W/O/W double emulsions are difficult to prepare on an industrial scale because they are thermodynamically unstable (Garti et al., 2004b; Hino et al. 2001; Gerald, 2007; Su et al., 2008) during the storage and on exposure to different environmental stresses for instance; mechanical forces, thermal processing, freezing or dehydration. These processes can lead to leakage of the internal aqueous droplets (W) in turn will affect the stability of the emulsion (Sapei et al., 2012). ***Oil-in-Water-in-Oil (O/W/O) emulsions:*** they are systems in which the primary emulsion, (O/W), is dispersed in the secondary oil phase. In other words, O/W/O emulsions are systems in which water droplets are enclosed by an oil phase, which in turn these water droplets surrounds one or more oil droplets (Vladisavljević, 2016).

Formulations of Multiple Emulsions

- **Oil Phase**

For any application of multiple emulsions, choosing the right oil is an important factor to be considered. For example, the oil phase to be employed in a pharmaceutical or food emulsion must be nontoxic. The numerous oils of vegetable origin (soybean, sesame, peanut, sunflower, etc.) are good choice for food applications and for pharmaceutical emulsion they are acceptable if purified correctly. Refined hydrocarbons such as light liquid paraffin, squalane, as well as esters of fatty acids have also been used in double emulsions. Oils derived from vegetable sources are biodegradable, whereas those based on mineral oils are only removed from the body very slowly. As a general rule, mineral oils produced more stable multiple emulsions (W/O/W) than vegetable oils (Florence and Whitehill, 1982).

- **Nature and Concentration of Emulsifying Agents**

Two different emulsifiers (lipohilic and hydrophilic) are necessary to form a stable emulsion. In general, for a W/O/W emulsion the optimal HLB value is in the range 2-7 for the primary surfactant and in the range 6-16 for the secondary surfactant. The concentration of the emulsifiers can also be varied. Applying small amount of emulsifier may result in unstable systems, while excessive amount of emulsifier may lead to toxic effects and can even cause destabilization. An excess of

lipophilic surfactant can cause the inversion of W/O/W emulsion to single O/W emulsion.

- **Phase fraction**

It is very important to have suitable order of phase addition while formulation and dispersed phase should be added slowly into the continuous phase for the formulation of a stable multiple emulsion. An optimal (22-50%) internal phase volume can be used for the emulsion formulation. Very high phase volume fraction (70-90%) had also been reported to produce a stable multiple emulsion (Prajapati et al., 2013).

1.2.2. Preparation Methods of Multiple Emulsions

Multiple emulsions can be formed by re-emulsification of the primary emulsion or when an emulsion inverts from one type to another, for example W/O to O/W or the opposite. However, in latter case the amount of internal dispersed phase created is small and such systems are probably of little industrial applications especially in therapeutics. Thus, for experimental studies, multiple emulsions are best prepared by re-emulsification of primary emulsion (Okochi and Nakano, 2000; Vladisavljević *et al.*, 2014).

- **Two-Step Emulsification Method**

In 1976, Matsumoto et al. have first proposed the development of multiple emulsions by two-step method (Matsumoto et al., 1976). This is the most common method used to prepare multiple emulsions because it is straightforward and gives high reproducibility of the emulsions (Garti and Bisperink, 1998; Garti and Lutz, 2004; Silva et al., 2016). This method involves two stages:

Primary stage: which includes preparing a primary emulsion, W/O in the case of making W/O/W emulsions, using high energy input, to attain fine inner droplets, and an appropriate hydrophobic emulsifier to stabilize the internal interface between the water phase and the oil phase in the primary emulsion.

Secondary stage: this stage involves re-emulsifying the primary emulsion with an additional aqueous phase containing an appropriate hydrophilic emulsifier to stabilize the external interface of the O/W emulsion and produce the final multiple emulsion, W/O/W. This stage usually carried out without using high energy input to avoid rupture of the internal droplets.

❖ Modified Two-Step Emulsification Method

Okochi and Nakano, 2000 reported a modified two-step emulsification technique for the preparation of W/O/W emulsion. This method is different from the two-step method in two points: the first point is; sonication and stirring are used to obtain fine, homogenous and stable W/O emulsion. While the second point is; the continuous phase is added to the dispersed phase to prepare W/O/W emulsion, see Figure 1.11. Furthermore, the composition of internal phase-oily phase-external phase is fixed at a ratio of 1:4:5, which produces most stable formulation as reported for most of W/O/W emulsions.

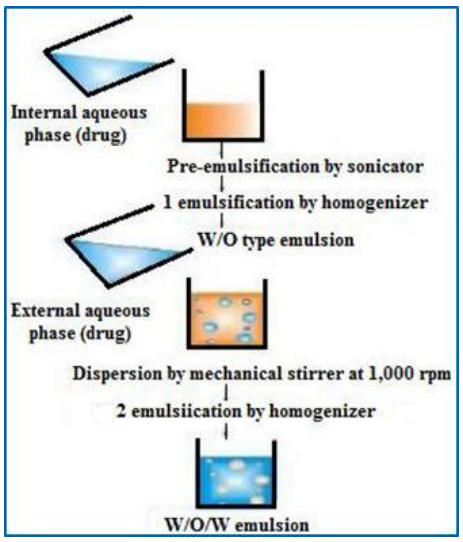


Figure 1.11. Modified two-step emulsification Technique (Prajapati et al., 2013).

❖ One Step Method (Phase inversion technique)

Preparation of W/O/W multiple emulsions which include intense mechanical agitation of the water phase containing a hydrophilic surfactant and an oil phase containing a large amount of hydrophobic surfactant. During this method, the volume concentration of dispersed phase increases gradually which subsequently leads to the development of multiple emulsions, see Figure 1.12. The steps of preparation of multiple emulsions using this method are described below:

i. First, a defined volume of the oil phase is placed in a vessel of pin mixer.
ii. Second, an aqueous solution of emulsifier is then added continuously to the oil phase in the vessel at a rate of 5ml/min, while the pin mixer rotates at 88 rpm at room temperature.

iii. Once the volume fraction of the aqueous solution exceeds 0.7, the oil phase replaced by the aqueous phase having an amount of the vesicular globules between the oil droplet causing phase inversion that leads to the formation of W/O/W double emulsion (Garti and Aserin, 1996).

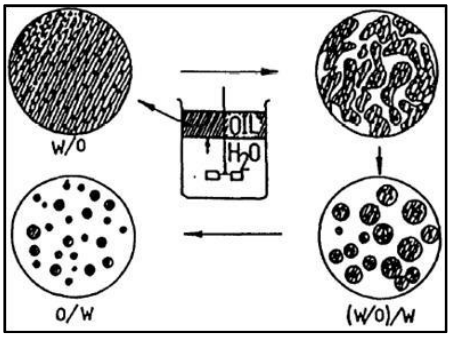


Figure 1.12. A possible sequence of events leading to the final formation of an o/w emulsion via a transient (W/O/W) emulsion, when the hydrophilic surfactant is initially located in the oil phase (Garti and Aserin, 1996).

❖ Membrane Emulsification Method

Membrane emulsification (ME) allows both a low size polydispersity of outer drops and high encapsulation yield of inner drops, because uniform drops can be formed even under quiescent conditions (Vladisavljević, 2016).

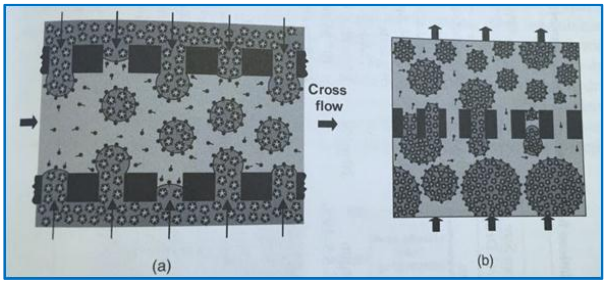


Figure 1.13. Different methods used to prepare multiple emulsions (Aserin, 2007).

The common membranes used in ME are Shirasu porous glass (SPG) (Vladisavljevi´c, Shimizu, & Nakashima, 2005). Hydrophilic membranes are needed to prepare W/O/W emulsions and hydrophobic membranes for O/W/O emulsions. A primary submicron emulsion is usually prepared by micro fluidisation, sonication, or high-shear mixing, and then ME is used for the secondary emulsification step. The concentration of inner phase is typically between 30 and 50 vol%, and the membrane pore size must be no less than twice the diameter of the internal drops (Vladisavljević, 2016).

In this process to prepare W/O/W emulsions, a W/O emulsion (dispersed phase) is added to an external aqueous phase (a continuous phase) with a constant pressure through a hydrophilic Porous Glass Membrane, see Figure 1.13. This method results in droplets with much less shear to help to achieve a high entrapment efficiency and monodispersity (Gladman, 2003). The drop size of the W/O/W emulsion can be controlled by the proper selection of porous glass membrane. W/O/W emulsions prepared using Shirasu Porous Glass (SPG) membranes were used for hepatic arterial chemotherapy. First, the sonication is used to make the primary W/O submicron emulsion. The anticancer drug was introduced in the water phase, and the oil phase was composed of iodized poppy seed oil with a hydrophobic surfactant (polyoxyethylene hydrogenated castor oil). The emulsion was made through the hydrophilic SPG membrane, which had a mean pore size 20µm and the previously obtained W/O emulsion as dispersed phase. The clinical trials have shown very promising results, since the multiple drug emulsion was effective in contracting the liver cancer texture when introduced directly into the liver via the hepatic artery (Higashi et al., 1999). This example shows that the Membrane Emulsification technique has much higher encapsulation efficiency than other preparation methods and, therefore, it is a critical approach to be used for the controlled release of drugs.

- **Solvent Evaporation Method**

This method is widely used for entrapping water-soluble compounds in particular for encapsulating proteins and peptides in W/O/W emulsions for sustained release applications and to prepare biodegradable hydrophobic microspheres (range size of 10 to 100µm) containing hydrophilic medicines (Garti and Bisperink, 1998; Aserin, 2007).

During this method, the internal aqueous phase is first prepared, contains water and the drug. Then, this mixture is added to the oil phase under shear to produce an oil phase comprising the polymer and the solvent, W/O emulsion. This W/O emulsion is then added to the external aqueous phase (water containing sufficient surfactant). The latter mixture is then homogenised to form the multiple emulsion W/O/W. The final step is the evaporation of the solvent, resulting in microparticles on the emulsion. The microspheres made by this method offer high capacities, stability for storage and instant reconstitution and controlled release (Garti and Bisperink, 1998), see Figure 1.14.

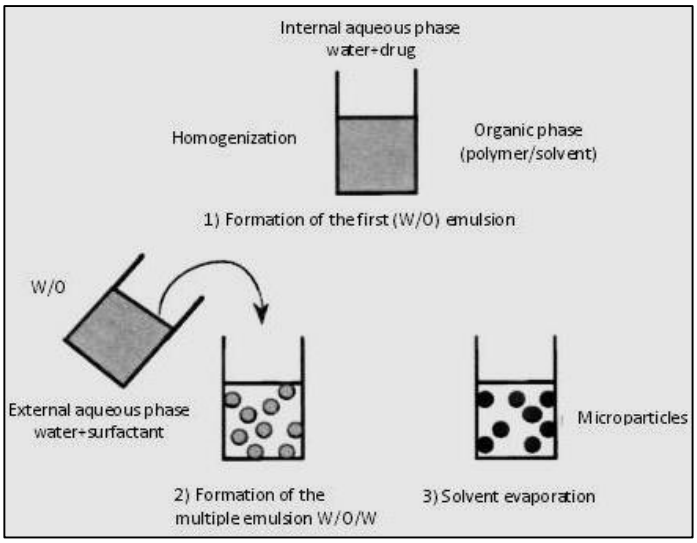


Figure 1.14. Scheme of the Solvent Evaporation Method to prepare Multiple Emulsions (Garti and Bisperink, 1998).

❖ Micro Channel Emulsification

The significant advantage in using microfluidic devices is the fact that they create emulsions in a single step, which leads to a precision control of the outer and the inner drop sizes and of the number of droplets encapsulated in each larger drop (Vladisavljevic and Williams, 2007).

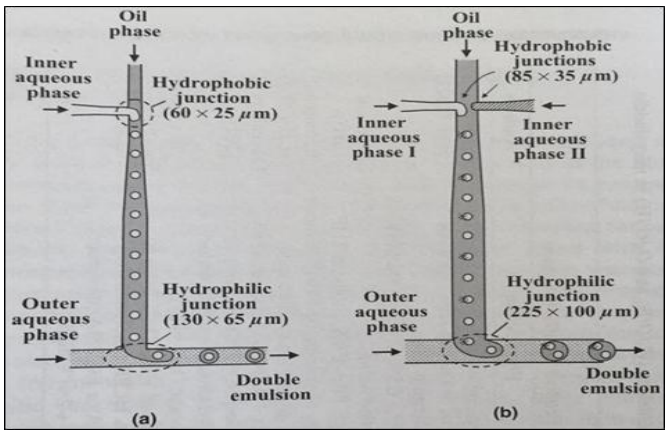


Figure 1.15. Double emulsion production with a Microfluidic Device using two T-junctions in series (Aserin, 2007).

One method to prepare monodisperse multiple emulsions consists in using T-junctions in series with different surface wettabilities and different sizes (Aserin, 2007; Vladisavljević *et al.*, 2014). The inner droplets formed in the smaller junction and outer droplets on the larger one. A silane-coupling agent could be used in any junction to modify it and make it hydrophobic. To prepare W/O/W

emulsions, first, the primary W/O emulsion should be prepared by homogenizing a mixture of water and oil phase. The second step is to force this emulsion through a microchannel device into a second water phase containing a suitable emulsifier for oil phase stabilization.

Figure 1.15, shows the production of a multiple emulsion with two T-junctions in series. The part ‘a’ corresponds to the production of a W/O/W emulsion in which each outer drop contains a single inner droplet. On part ‘b’ the production of a W/O/W emulsion in which each outer drop contains two inner droplets consisting of different aqueous phases is shown. An upstream cross junction could be used to generate different inner droplets.

Vladisavljević et al (2018) investigated the stability of oil-in-water emulsions by microchannel (step) emulsification using two cross flow silicon chips consisting of microchannels. Their results indicated that microchannel (step) emulsification technology in combination with solvent evaporation is very suitable for long-term production of synthetic biodegradable polymer particles. The particle size can be precisely adjusted by the microchannels and terraces and the droplet throughput can be controlled by the total number of microchannels.

Nabavi et al (2015) developed a three-phase axisymmetric numerical model based on Volume of Fluid–Continuum Surface Force (VOF–CSF). This model was developed to perform parametric analysis of compound droplet production in three-phase glass capillary devices that combine co-flow and counter current flow focusing. They successfully generated of core–shell and multi-cored double emulsion droplets in dripping and jetting (narrowing and widening) regime.

1.2.3. Multiple Emulsions by Two-step Emulsification Method

This section provides an overview of the research works conducted regarding the formation of W/O/W multiple emulsions by two-step emulsification method. Multiple emulsions are characterized by the average drop size and the percentage of entrapped materials in the primary phase. Multiple emulsions have been proposed to have numerous uses. Some of the main proposed mechanisms for release of materials entrapped in the primary phase are presented, along with a discussion of the factors that affect and control the release. The literature review deliberates on the possible factors that affect the stability of multiple emulsions and related to the materials used in the work of this thesis.

1.2.3.1. Stability of Multiple Emulsions

The biggest problem concerning the stability of this type of emulsions is the presence of two thermodynamically unstable interfaces. Two different emulsifiers are necessary for their stabilization: one with a low HLB for the internal W/O interface and the second one with a high HLB for the external O/W interface (Rajesh et al., 2012). The instability in multiple emulsions, W/O/W, is represented by the release of electrolytes/active materials from internal to external phase through the oily layer by a different mechanism. Multiple emulsions were found to be thermodynamically unstable, even more than conventional emulsions, for the following reasons (Lutz et al., 2009):

- Their relatively large droplet size, as well as the difficulty in controlling the release of entrapped compounds.
- Besides, water molecules may be transported across the oil layer from the inner to the outer phase or in the opposite direction, depending on the osmotic pressure gradient between the two aqueous phases.
- Water transported from the inner phase empties the oil globules, and inverts W/O/W double emulsion into single O/W emulsion.
- Other instabilities in W/O/W double emulsions are related to creaming of the oil globules in the continuous aqueous phase.

How does release occur?

There are four possible mechanisms leading to instability of W/O/W double emulsions (O'Regan and Mulvihill, 2010), these are:

- Migration/diffusion of water and water soluble materials from the internal and external aqueous phases through the oil phase via reverse micellar transport (O'Regan and Mulvihill, 2010; Rajesh et al., 2012).
- Coalescence of the internal aqueous droplets.
- Coalescence of the oil droplets.
- Rupture of the oil film, which separates the internal and external aqueous phases, resulting in the loss of the internal aqueous droplets.

The above mechanisms can be classified as two main routes for release of an ionic electrolyte from inner aqueous phase to the external aqueous phase, as described below:

1. *Diffusion via inverse micelles*

The first mechanism, diffusion of ions through inverse micelles, occurs via the following stages:

i) Reverse micelles including ions are formed by disintegration of ions from the internal water droplets. (The ions have to cross water – oil interface, diffuse through oil phase, cross the micelle interface and then diffuse into the core of micelles.

ii) Micelles have to diffuse through the oil phase towards the oil-external drop interface.

iii) Micelles are unloaded with ions at the interface.

2. *Membrane rupture, coalescence or exit*

For the second mechanism, however, release, via membrane rupture, is related to the fraction of the water phase that has entered the external continuous phase. The initial fraction of the dispersed phase is set by the emulsion composition. However, two phenomena contribute to increasing the effective dispersed phase, these are:

i) *Osmosis driven instability*

Multiple emulsions tend to undergo swelling due to osmotic pressure induced by the differences between the concentrations of substances in the most inner and outer phases. For W/O/W emulsions, water can go from the continuous phase to inner water droplets. Usually water-soluble solute such as glucose is added to the external water phase to balance the osmotic pressure induced by the presence of salt in the internal water phase. However, this may affect the final properties of the resulting emulsions.

ii) *Inclusion*

Multiple emulsions are mixtures of two types of emulsions. The oil-soluble surfactant tends to stabilise water-in-oil emulsions, W/O. When the primary W/O emulsion is added to the secondary water phase to form W/O/W multiple emulsions, it is likely that the oil phase includes some water from the external water phase. The mechanism of inclusion has been attributed to drop deformation. The inclusion combined with possible coalescence of drops inside oil drops make tracking even more complicated. This suggests that the change in phase fraction may not be directly related to the release.

Strategies to improve the stability of W/O/W multiple emulsions

A W/O/W emulsion, upon keeping, may show phase separation due to density difference between the oil phase and the aqueous phase or due to large size of multiple drop. To improve the stability of W/O/W multiple emulsions; numerous strategies have been proposed for this reason such as: decreasing the viscosity difference between aqueous and oil phase by increasing the viscosity of the external aqueous phase (Hattrem et al., 2014; Özer et al., 2000; Kanouni et al., 2002; Benna et al., 2008); the addition of different emulsifier mixtures to the aqueous phases and/or to the oil phase (Shima et al., 2004; Su et al., 2006); increasing the concentration of secondary surfactant; the addition of oil-insoluble electrolytes to the internal aqueous phase in order to balance the Laplace and osmotic pressures between the internal and the external aqueous phases (Garti, 1997b; Kanouni et al., 2002; Rosano et al., 1998); the solidification of the oil phase; the modification of the solubility and polarity of the oil phase to reduce its solubility in the water (Tedajo et al., 2001) and the combination of stabilizers (Benna et al., 2008) and biopolymers in the external aqueous phase (Allouche et al., 2003; Benichou et al., 2004; Benichou et al., 2007).

1.2.3.2. Factors affecting stability of W/O/W multiple emulsions

There are many factors influence the release and hence the stability of W/O/W multiple emulsions (Panagopoulou et al., 2017; Su et al., 2008):

- The preparation method.
- The composition of the emulsion, i.e. the nature of oil phase, type of emulsifiers and the nature of entrapped materials.
- The presence of electrolytes.
- Other parameters such as shear/agitation, phase volume, the concentration of components also need to be considered.

Much research works has been carried out on optimizing the nature of the oils, the weight fractions of the water and the oil, and the surfactants the concentration and the nature of for the sake to overcome instability- and release problems in multiple emulsions, below is a review of some of these works.

Effect of surfactant type and concentration

To produce W/O/W, multiple emulsions by two-step method at least two kinds of surfactants must present. One to be a hydrophobic surfactant, with low HLB,

used to prepare the primary emulsion and another one to be a hydrophilic surfactant, with high HLB, used to make the secondary emulsion (W/O/W). To prepare stable multiple emulsions the right amount of surfactant is needed. It has been reported by Florence et al. (1985) that the proper range of the surfactant amount is between 1 to 10%. In addition, the physical–chemical properties of the surfactants at the O/W interface and the interactions among the surfactants dissolved in the aqueous phase were found to play a significant role in the stability of the emulsions (Kanouni et al., 2002). Thus, an increase in the concentration of hydrophilic surfactant in the external phase would destabilise multiple emulsion the system becomes less stable (Matsumoto et al., 1976; Csóka and Erõs, 1997). This is because when the concentration of lipophobic surfactant in the outer phase is higher than its Critical Miceller Concentration, lipophilic surfactant in the primary emulsion might solubilise in the lipophobic surfactant and move it to the continuous phase as a result the concentration of lipophilic surfactant decreases at the interface of the primary emulsion, W/O, which causes the rupture of oil layer and the release of internal drops (Csóka and Erõs, 1997).

In 1996 Apenten and Zhu, introduced the effective minimum concentration (EMC) to describe the minimum amount of surfactant required to produce a stable multiple emulsion, W/O/W. They used non-ionic low-molecular weight emulsifiers, Span 80 and Tween 20, to stabilize multiple emulsions. They monitored the stability of multiple emulsions and yield as a function of surfactant concentration required to form a 'calculated monolayer'. The emulsifier monolayer calculations are based on the emulsifier-specific surface area, the emulsifier surface excess, and the emulsion interfacial area. They concluded that the concentration of the hydrophilic emulsifier at the oil/water interface, Tween 20, has no effect on the production and stability of the multiple emulsions. Therefore, a low concentration of the hydrophilic emulsifier is used. Multiple emulsions can be produced without the hydrophilic emulsifier. However, such emulsions prepared in the absence of a hydrophilic emulsifier, will be coarse and not very stable.

Furthermore, the stability of multiple emulsions could be associated with the concentration of the lipophilic surfactant. It was indicated that the swelling capacity of the oil droplets considerably increases as the concentration of the lipophilic surfactant increases, and the more the oil droplets swell, the less the water-soluble drug releases (Jager-Lezer et al., 1997). This influence explained

by two different mechanisms. The first mechanism, strengthens the second interface by the gradual migration of the lipophilic surfactant. During the second step of multiple emulsion preparations, lipophilic surfactant molecules can diffuse from the first to the second interface, where they produce a synergistic effect resulting in membrane strengthening. The second mechanism involves a delay in the in the aqueous droplet coalescence. In the course of swelling of the oil droplets, the lipophilic surfactant molecules, which are in excess in the oily phase, can diffuse to the first interface to fill up free spaces caused by swelling, when required. However, using an excess of hydrophilic surfactant could destabilise the emulsion (Jager-Lezer et al., 1997). In contrast, it was suggested that low amount of lipophilic surfactant is needed to prepare the primary, W/O, emulsion in such a way that the W/O interface is saturated (Kanouni et al., 2002). In a study to investigate the efficiency of the emulsifiers or stabilizers in multiple emulsions; Panagopoulou et al., 2017 prepared multiple emulsions W/O/W using polyglycerolpolyricinoleate (PGPR) as the lipophilic emulsifier and whey protein isolate (WPI) and Bacterial cellulose (BC) as the hydrophilic stabilizers. They found out that, the droplet size as well as viscosity, is highly dependent on the concentration of the emulsifier or the stabilizer used. Using high BC or PGPR concentrations produce small droplet sizes and higher viscosity levels. However, the incorporation of WPI in the emulsions, alone or in mixture with BC, resulted in bigger particle sizes which in turn results in phase separation of the emulsions (Panagopoulou et al., 2017).

It is well known that an emulsifier with an optimum HLB value and with chemical properties compatible with emulsion ingredients is necessary to achieve a stable emulsion. Schmidts et al. (2010) determined the ideal HLB value for the hydrophilic emulsifier in multiple W/O/W emulsions. They reported that to stabilise the primary emulsion, the required HLB should be ranged between 4.3–4.7 when paraffin oil is used as the oil phase. They also found that the optimum HLB value determined for hydrophilic emulsifiers for multiple, W/O/W, emulsions containing 4% lipophilic surfactant of HLB = 4.3 and 1% of a hydrophilic surfactant to be ranged between 15 and 15.5. Tween 80 and Span 80 are very often used in multiple W/O/W emulsions because of their similar chemical structure. In the majority of cases the most stable emulsions, in particular, are formed when both emulsifying agents are of the same hydrocarbon chain length. The greater stability of multiple emulsions containing Tween 80 can be related to

two factors; the chemical compatibility of Tween 80 and Span 80 and the better HLB value (Schmidts et al., 2009).

A range of HLB values of hydrophilic emulsifiers used to produce multiple W/OW emulsion is listed in Table 1.2. It is well-known that mixture of emulsifiers at the external phase have a potential influence on the stability of multiple emulsions (Garti and Bisperink, 1998). Kanouni et al. (2002) described that the interactions between the low and high HLB emulsifiers at the O/W interface should not destabilize the films. They reported that using a blend of two high HLB surfactants in the outer aqueous phase: an amphoteric surfactant such as Betaine and an anionic surfactant such as sodium lauryl ether sulfate would contribute to the stability of the film.

Table 1.2. HLB values of hydrophilic emulsifiers used for multiple W/OW emulsions (Schmidts et al., 2009).

Chemical structure	HLB value
Ester *Polyethoxylated sorbitan and fatty acid esters*	
Tween 80	15
Tween 20	16.7
Polyethoxylated derivatives of stearic acid	
PEG-20 stearate	15
PEG-40 stearate	17
PEG-40/50 stearate	16.9
PEG-100 stearate	18.8
Sucrose ester	
Sucrose palmitate	15
Ether *Polyethoxylated fatty alcohols*	
Laureth-20/23	16.9
Steareth-10	12.4
Steareth-20	15.3
Cetostereath-12	13.4
Cetostereath-20	15.7
Cetostereath-25	16.2
Cetostereath-30	17
Oleth-20	15.5
Polymer	
Poloxamer 407	22

Effect of oil fraction

Bonnet et al. (2010) investigated the effect of the oil globule fraction and magnesium chelation on release profiles from multiple W/O/W emulsions. They demonstrated that the oil globule fraction is an efficient way to improve the release of the encapsulated species in the internal aqueous droplets. Firstly, by reducing the relative volume of the outer phase, the total amount released at equilibrium decreased. Secondly, the permeation coefficient tended to decrease with the extent of magnesium required, and the effect was increasingly pronounced as the globule fraction increased.

Effect of electrolyte

Electrolytes have a significant effect on the release of active ingredients from the internal drops to the secondary phase and hence the stability of multiple emulsions (Rosano et al., 1998; Kanouni et al., 2002; Spyropoulos et al., 2011). The presence of electrolytes lowers the attractive force between water droplets and therefore reducing collision frequency (Márquez et al., 2010; Frasch-Melnik et al., 2010a; Frasch-Melnik et al., 2010b; Matos et al., 2014). Therefore, to ensure inner droplets stability salts should be added to the internal aqueous phase. Aronson and Petko (1993) described that electrolytes could increase emulsifier adsorption density at the oil/water interface and reduce interfacial tension in W/O emulsions. On the other hand, Scherze et al. (2006) showed that addition of NaCl to the dispersed phase of PGPR-stabilized W/O emulsions is essential in preventing dispersed water droplet coalescence. Salt added to the inner aqueous phase exerted an osmotic pressure that caused diffusion of water into the inner aqueous phase and increased W/O/W emulsion viscosity through an increase in the volume fraction of the primary W/O emulsion. This type of viscosity increase imposed a destabilizing effect because of the likelihood of rupture of the inner and multiple droplets (Jiao and Burgess, 2003).

It was reported that release increases as the concentration of NaCl decreases in the presence of 3% gelatin (Sapei et al., 2012), see Figure 1.16(A).

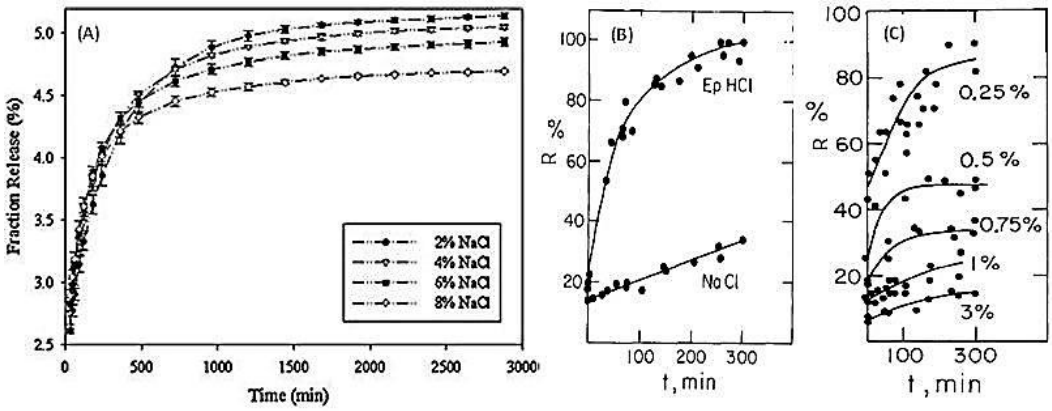


Figure 1.16. A) Release patterns of W/O/W multiple emulsions examined via conductivity, emulsions stabilized with 3% gelatine combined with various NaCl concentrations entrapped in the inner aqueous phase (Sapei et al., 2012); B) Release versus time for Ephedrine HCl (0.17 M) and NaCl(O.17 M); C) Release versus time at different concentrations NaCl (Magdassi and Garti, 1984).

The size of the oil drops increases with the increase in the NaCl concentration. This is caused by the high osmotic pressure gradient experienced between the

two aqueous phases, suggesting greater water diffusion from the external to the internal aqueous phase. The difference in the osmotic pressure between the internal aqueous phase and the external aqueous phase affects the stability of multiple emulsions. A large osmotic pressure gradient can cause the inner droplets to shrink or swell as a result of diffusion of water molecules across the oil layer. When the osmotic pressure is higher in the external aqueous phase than the internal aqueous phase, water molecules diffuse from the internal to the external aqueous phase causing internal drops to shrink. When the osmotic pressure is higher in the internal aqueous phase water molecule from the external aqueous phase diffuse to the internal aqueous phase until equilibrium is reached, this causes the swelling of internal drops.

Some researcher reported that release is affected by the nature, hydrophobicity, of the electrolyte and by its concentration (Magdassi and Garti, 1984; Hattrem et al., 2014). Figure 1.16(B) shows the effect of the electrolyte nature, ephedrine hydrochloride, and sodium chloride, on release. From this graph, it can be seen that Ephedrine hydrochloride released faster than sodium chloride. The authors also reported the effect of NaCl concentration on time; see Figure 1.16(C). The release was decreasing as the concentration of NaCl increasing. The authors explained that the reason for such behavior is not due to the interfacial effects of the two electrolytes, as described by Florence and Whitehill (Florence and Whitehill, 1982), but it is due to the differences in their hydrophilicity, or their state of hydration.

In another study by Lutz et al. (2009) similar findings were reported. In this study, NaCl was used as an inorganic salt, and sodium ascorbate was used as organic. Sodium ascorbate behaves similarly to NaCl but is released faster. Ascorbate is a relatively larger ion and less hydrated than the chloride ion, and therefore, it can be transported easier and quicker through the oil membrane. Sodium ascorbate was released faster and to a greater extent than NaCl. Later in 2002, Kanouni et al found the same assumption.

Effect of viscosity of the phases

Several studies had attempted to slow down creaming of multiple emulsions by adding a thickener to either of the phases to increase their viscosity. However, a high viscosity of the external continuous phase causes a crucial problem on the fabrication of the multiple emulsions because it can cause the breakdown of the

oily globules especially because of the relatively high diameter of oily globules (Benna et al., 2008). In an investigation of the preparation and stability of W/O/W emulsions done by Kanouni et al (2002), it was reported that the presence of a thickener in the external phase is necessary in order to reach a viscosity ratio between the W/O and W phases, allowing dispersion of the viscous primary emulsion into the external aqueous phase. The thickener serves as a dispersant and therefore prevents phase separation. Similarly, the nature of the oil can significantly affect the behavior of the system concerning properties like viscosity or water solubility (Florence and Whitehill, 1982; Garti, 1997a).

A mixture of oils can be used to vary the viscosity of the oil phase to control the movement of the solute across the oil membrane (Sinha and Kumar, 2002). Selection of the oil phase can affect various emulsion parameters such as release profile, drop size and emulsion stability. W/O/W emulsions prepared with high viscosity oils tend to have larger drop size. A positive correlation between the interfacial tension at the oil - water interface and the internal droplet size has been proposed. Viscose oil produces W/O/W emulsions which are more stable in term of percentage breakdown (Sinha and Kumar, 2002).

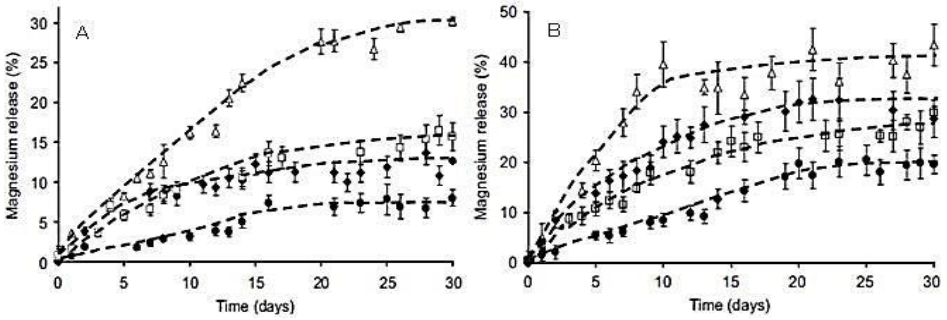


Figure 1.17. Magnesium release for W/O/W emulsions based on olive oil (♦), olein (□), miglyol (Δ) and rapeseed oil (●) for 30 days, and stored (A) at 4°C and (B) at 25°C (Bonnet et al., 2009).

In a study by Bonnet et al. (2009), water-in-oil-in-water (W/O/W) emulsions were formulated to investigate the effect of the oil nature on the release rates of magnesium encapsulated in the inner droplets. Emulsions were prepared using a two-step emulsification procedure in the presence of Polyglycerol polyricinoleate and sodium caseinate as lipophilic and hydrophilic emulsifiers, respectively. Different oils were employed as the oil phase, rapeseed oil, olive oil, olein, and miglyol, see Figure 1.17. In this study, the stability of multiple emulsions and ion release were monitored over time. The authors reported that magnesium release

was highly dependent on the oil type used in the W/O/W emulsion formulation: the higher release was obtained for the oils characterized by, the lower viscosity. Magnesium release was not due to droplet–globule coalescence but rather to diffusion and/or permeation mechanisms with a particular rate that was not constant over time. However, controversial results were found with other studies showing that the viscosity of the oil phase may be used to control the diffusion rates of solutes across the oil membrane (Florence and Whitehill, 1982; Garti, 1997a). While other studies found comparable kinetic evolution regardless of the oil nature (Pays et al., 2002).

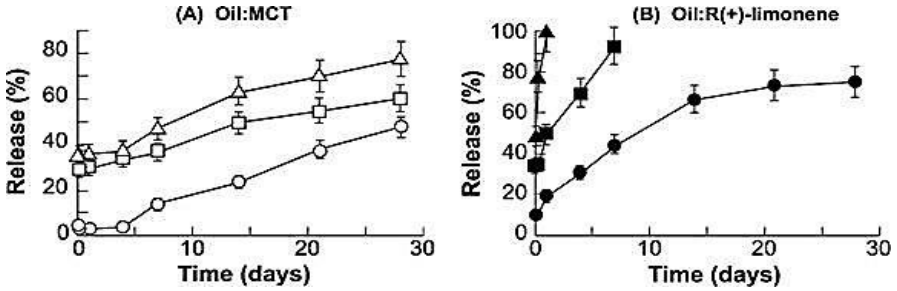


Figure 1.18. Release profile (%) of NaCl from the inner aqueous phase, at room temperature from DEs, stabilized by WPI/C63 4/0.5 (w/w), at pH=6 with different NaCl concentrations: 1 wt.% (Δ, ▲), 1.5 wt.% (□, ■), and 4.4 wt.% (○, ●). The primary emulsion consists of 10 wt.% PGPR, 60 wt.% oil phase, (A) MCT and (B) R(+)-limonene, and 30 wt.% aqueous phase (Lutz et al., 2009).

In another study, it was reported that the oil type determines the mechanism and the rate of the release (Lutz et al., 2009). In this study, W/O/W, multiple emulsions stabilized by charged soluble complexes of whey protein isolate (WPI) and modified pectins were investigated in relation to their stability and the release of two types of electrolytes using two different oils, Medium chain triglyceride (MCT) and R (+)-limonene. The influence of NaCl concentration and the oil type on the release rate is demonstrated in Figure 1.18. This figure shows that in both multiple emulsions, based on MCT and R (+)-limonene, the release rate decreases as the NaCl concentration increases. Although the release of NaCl, for both oils, has a similar trend there are significant differences between them. MCT- multiple emulsions exhibited a delay time of 5 days until the great release of the NaCl to the outer phase occurred, whereas in the R (+)-limonene-multiple emulsions no delay time was noticed. Also, the release rate in MCT- multiple emulsions was slower than the release rate in R(+)-limonene- multiple emulsions. The diffusion mechanism for MCT-DEs is diffusion controlled of salt and water in opposite directions while the R (+)-limonene-DE is stability-controlled (breakdown

or "thinning the liquid film") since the oil is very hydrophobic the transport is insufficient and very slow. The R(+)-limonene release mechanism is not a breakdown of the droplets since this would involve a release of NaCl with osmotic pressure gradient and the diffusion of water into the inner phase should have been increased with a greater rate of release.

Effect of stirring speed

It is well known that high agitation disturbs the stability of the emulsions and, consequently, results in an unstable system due to an increase in the surface area. As a result, with more homogenization time, the emulsions dissipate quickly. Usually, high shear speed provided during primary emulsification while a lower shear rate provided during second emulsification. The low shear rate is preferred during second emulsification stage to prevent possible rupture of the multiple emulsion droplets. This rupture will cause a release of the active materials entrapped in the inner phase during the homogenization which in turn lead to invert the W/O/W emulsion into a single O/W emulsion and to reduce yields of preparation (Garti and Aserin, 1996). On the other hand, slow or gentle stirring will form large multiple emulsion droplets with limited stability (Florence and Whitehill, 1981).

1.3. Hydrogel Particles

1.3.1. Introduction

Hydrophilic gels that are usually referred to as hydrogels are networks of polymer chains that are sometimes found as colloidal gels in which water is the dispersion medium (Ahmed et al., 2013). They have a three-dimensional configuration capable of holding large amounts of water or biological fluids (Peppas et al., 2000). Therefore, they possess a high degree of flexibility. Under physiological conditions, they are able to retain a large amount of water or biological fluids and are characterized by a soft rubbery consistency similar to living tissues, making them an ideal substance for a variety of applications (Ullah et al., 2015). Their ability to absorb water arises from hydrophilic functional groups attached to the polymeric backbone, while their resistance to dissolution arises from crosslinks between network chains (Mathur et al., 1996).

Hydrogels have a highly porous structure that can easily be tuned by controlling the density of cross-links in the gel matrix and the affinity of the hydrogels for the aqueous surroundings in which they are swollen. Their porosity also permits loading of drugs into the gel matrix and subsequent drug release at a rate dependent on the diffusion coefficient of the small molecule or macromolecule through the gel network (Hoare and Kohane, 2008).

Due to its biocompatibility and requirement for mild gelation conditions, sodium alginate is an attractive and cost-effective material for biomedical applications. Thus, sodium alginate and modified sodium alginate hydrogels have been used for tissue engineering, drug delivery, cell encapsulation and wound healing applications (Le Goff et al., 2015; Ahmad et al., 2006).

Classification of hydrogel products

Hydrogel products can be classified on different bases as detailed below:

1. Classification based on source: Hydrogels can be classified into two groups based on their natural or synthetic or hybrid origins, depending on the source of the constituting polymers (Buwalda et al., 2014).
2. Classification according to polymeric composition: The method of preparation leads to formations of some important classes of hydrogels. These can be homopolymeric hydrogels, copolymers hydrogels or multipolymer Interpenetrating polymeric hydrogel (IPN).

- ❖ Homopolymeric hydrogels are referred to polymer network derived from a single species of monomer, which is a basic structural unit comprising of any polymer network. Homopolymers may have cross-linked skeletal structure depending on the nature of the monomer and polymerization technique.
- ❖ Copolymeric hydrogels are comprised of two or more different monomer species with at least one hydrophilic component, arranged in a random, block or alternating configuration along the chain of the polymer network.
- ❖ Multipolymer Interpenetrating polymeric hydrogel (IPN), an important class of hydrogels, is made of two independent cross-linked synthetic and/or natural polymer component, contained in a network form. In semi-IPN hydrogel, one component is a cross-linked polymer, and another component is a non-cross-linked polymer.

3. Classification based on configuration: Hydrogels can be classified depends on their physical structure of the networks as Crystalline, Crystalline phases, amorphous (non-crystalline), Semi crystalline (complex mixture of amorphous) (Ahmed, 2015; Hamidi et al., 2008), hydrogen-bonded structures, super molecular structures, hydro colloidal aggregates (Madolia and Sheo, 2013).
4. Classification based on the type of cross-linking: Hydrogels can be chemically cross-linked by covalent bonds, physically cross-linked by non-covalent interactions or cross-linked by a combination of both (Buwalda et al., 2014). Chemically cross-linked networks have permanent junctions, while physical networks have transient junctions that arise from either polymer chain entanglements or physical interactions such as ionic interactions, hydrogen bonds, or hydrophobic interactions (Chauhan et al., 2012).
5. Classification based on physical appearance: Hydrogels appearance as matrix, film, or microsphere depends on the technique of polymerization involved in the preparation process (Ahmed, 2015).
6. Classification according to network electrical charge: Hydrogels may be categorized into four groups on the basis of presence or absence of electrical charge located on the crosslinked chains: Nonionic (neutral); Ionic (including anionic or cationic); Amphoteric electrolyte (ampholytic) containing both acidic and basic groups; Zwitterionic (polybetaines) containing both anionic and cationic groups in each structural repeating unit.

Hydrogel-forming natural polymers include proteins such as collagen and gelatine and polysaccharides such as starch, sodium alginate, and agarose. Synthetic polymers that form hydrogels are traditionally prepared using chemical polymerization methods.

1.3.2. Synthesis of Hydrogel Particles

Hydrogels can be prepared from either synthetic polymers or natural polymers. They may also be prepared using a mixture of both synthetic and natural polymers (Ahmed, 2015). The synthetic polymers are hydrophobic in nature and chemically stronger compared to natural polymers. Their mechanical strength results in slow degradation rate, but on the other hand, mechanical strength provides the stability as well. These two opposite properties should be balanced through optimal design (Tabata, 2009).

A hydrogel particle is simply a hydrophilic polymeric network cross-linked in some fashion to produce an elastic structure. Thus, any technique which can be used to create a cross-linked polymer can be used to produce a hydrogel. Any of the various polymerization techniques can be used to form gels, including bulk, solution, and suspension polymerization. In general, the three integral parts of the hydrogels preparation are a monomer, initiator, and cross-linker. To control the heat of polymerization and the final hydrogels properties, diluents can be used, such as water or other aqueous solutions. Then, the hydrogel mass needs to be washed to remove impurities left from the preparation process. These include a unreacted monomer, initiators, cross-linkers, and unwanted products produced via side reactions Figure 1.19.

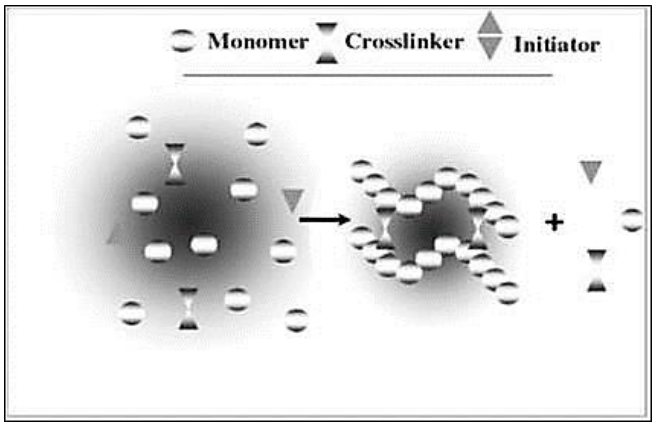


Figure 1.19. Schematic diagram of hydrogel preparation (Ahmed, 2015).

Bulk polymerization: Bulk hydrogels can be formed with one or more types of monomers. Usually, a small amount of cross-linking agent is added in any hydrogel formulation. The polymerization reaction is normally initiated with radiation, ultraviolet, or chemical catalysts. Bulk polymerization is the simplest technique which involves only monomer and monomer-soluble initiators. The high rate of polymerization and degree of polymerization occur because of the high concentration of monomer (Ahmed, 2015). The bulk polymerization of monomers to make a homogeneous hydrogel produces a glassy, transparent polymer matrix which is very hard. When immersed in water, the glassy matrix swells to become soft and flexible.

Solution polymerization/cross-linking: In solution copolymerization/cross-linking reactions, the ionic or neutral monomers are mixed with the multifunctional crosslinking agent. The polymerization is initiated thermally by UV-irradiation or by a redox initiator system. The presence of solvent serving as a heat sink is the major advantage of the solution polymerization over the bulk polymerization (Ullah et al., 2015). Phase separation occurs, and the heterogeneous hydrogel is formed when the amount of water during polymerization is more than the water content corresponding to the equilibrium swelling. Typical solvents used for solution polymerization of hydrogels include water, ethanol, water-ethanol mixtures, and benzyl alcohol (Kofinas et al., 1996).

Suspension polymerization or inverse-suspension polymerization: Dispersion polymerization is an advantageous method since the products are obtained as powder or microspheres (beads), and thus, grinding is not required. Since water-in-oil (W/O) process is chosen instead of the more common oil-in-water (O/W), the polymerization is referred to as ‘‘inverse suspension’’. In this technique, the monomer solution, containing the initiator, is dispersed in the non-solvent forming fine droplets (Madolia and Sheo, 2013). Since the dispersion is thermodynamically unstable, continuous agitation and the addition of a low hydrophilic–lipophilic- balance (HLB) stabiliser are required. The size and the shape of the particles are affected by the viscosity of the monomer solution, agitation speed, rotor design, and dispersant type (Ogata et al., 2006).

Grafting to a support: Hydrogels prepared by bulk polymerization have inherent weak structure. To improve the mechanical properties of a hydrogel, it can be grafted on a surface coated onto a stronger support. This technique that involves the generation of free radicals onto a stronger support surface and then

polymerizing monomers directly onto it. As a result, a chain of monomers is covalently bonded to the support. A variety of polymeric supports have been used for the synthesis of the hydrogel by grafting techniques (Qunyi and Ganwei, 2005).

Polymerization by irradiation: Ionizing high energy radiation, like gamma rays and electron beams, has been used as an initiator to prepare the hydrogels of unsaturated compounds. The benefits of radiation crosslinking are that the network formation is completed without a potentially toxic crosslinking agent and that there are no chemical functional groups in the crosslinked structure. Irradiation can be achieved either in bulk or in aqueous solutions. Irradiation in aqueous solution increases the mobility of the polymer chains and increases the probability of free radical crosslinking (Stringer and Peppas, 1996). Irradiation of an aqueous solution of a polymer at higher concentration is a powerful method for hydrogel synthesis (Burkert et al., 2007). Charles (1959) studied cross-linking of polymers initiated by high-energy radiation in detail. Krsko et al. (2003) used the focused electron beam of a field emission electron microscope for simultaneous crosslinking and patterning of poly (ethylene oxide) (PEO). Similar studies of the cross-linking were found elsewhere (Hegewald et al., 2005, 2006).

1.3.2.1. Synthesis of Alginate Hydrogel Particles

Sodium alginate is a naturally available anionic polysaccharide that is extracted from brown algae and can be precipitated into alginic acid at low pH (1.5– 3.5). Alginate can be crosslinked by ionic interactions, typically calcium Ca^{2+} divalent ions, at room temperature and physiological pH (Ullah et al., 2015). It has a mixture of unbranched copolymers structure containing different sequences of (1,4)-linked-ß-D-mannuronate (M) and a-L-guluronate (G) residues. The interaction between the cations and the carboxylic groups of the polysaccharide induces gelation, and it is believed that only G-blocks are involved in intermolecular cross-linking. The hydrogel physical properties, such as its porosity and stiffness, depend on sodium alginate composition (molecular weight distribution, G block/M block ratio, and sequence) and the stoichiometry of the sodium alginate with the divalent cation (Chan et al., 2011; Graulus et al., 2015).

It is well known that crosslinking agent has a pronounced effect on the release behavior of drugs from the crosslinked matrix. Crosslinking of sodium alginate, in general, is done with calcium chloride, resulting in formation of calcium alginate,

which is water insoluble. The crosslinking between sodium alginate and calcium chloride is achieved by the ionic interaction between Ca^{2+} ions and the carboxyl groups of the guluronic acid residues of two neighbouring alginate chains, resulting in the formation of a three dimensional network. When sodium alginate comes into contact with calcium ions (Ca^{2+}), see Figure 1.20. As soon as sodium alginate is added to a solution of calcium chloride, a gel forms as the sodium ions (Na^{+}) are exchanged with calcium ions (Ca^{2+}) and the polymers become cross-linked. The calcium ions are able to crosslink the alginate polymers because they can form two bonds, as opposed to monovalent ions such as sodium, which can only form one bond. The longer the alginate is in contact with the calcium chloride solution, the more rigid the gel will become, as more crosslinks are formed. Also, depending on the concentration of calcium ions (Waldman et al., 1998).

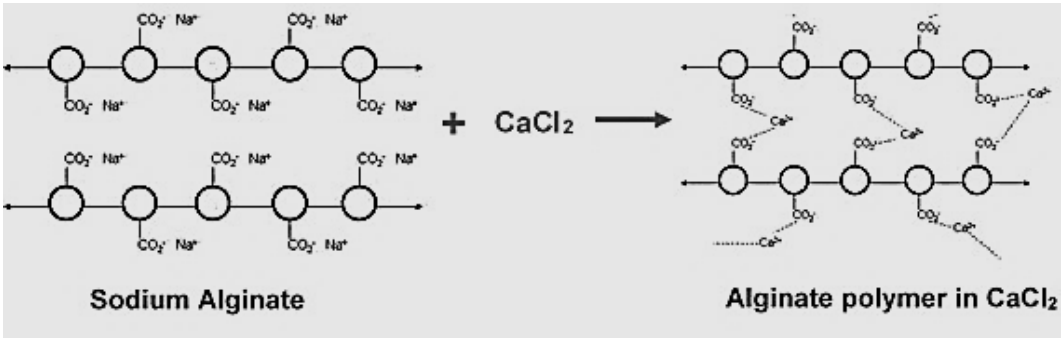


Figure 1.20. Alginate polymer in $CaCl_2$ solution (crosslinking) (Waldman et al., 1998)

Here the most common methods of producing alginate hydrogel particles are discussed:

Simple dripping (extrusion): Simple dripping is the most commonly used technique for producing alginate beads (Blandino et al., 1999; Lupo et al., 2014; Chan et al., 2011). However, producing beads with desired size and spherical-shaped requires some trial and error works on the liquid formulation and experimental set-up. The basis of this method involves the drop-wise extrusion of alginate droplets from a loaded syringe into a calcium gelling bath (typically calcium chloride) (Pereira et al., 2013; Jang et al., 2014). When the alginate solution flows out of the syringe opening, a droplet is formed at the needle tip. The alginate droplet grows in size until the droplet separates from the needle tip and fall into the gelling bath. Alginate macrogels produced by simple dripping are generally in the millimeter size range (1–2 mm) (Chan et al., 2011).

Gel particle size and morphology can vary depending on the viscosity of the alginate, diameter of the needle opening, exit flow rate of the alginate, and height of alginate exit point from the gelling bath (Blandino et al., 1999). Particle sphericity is influenced by the distance between the needle tip and the gelling bath as well as the flow rate of alginate (Chan et al., 2009).

Although extrusion by syringe is the simplest method of producing uniform alginate gel particles, the main limitations of this technique are the large size of beads formed and the difficulty of large scale production because beads are formed one by one (Lupo *et al.*, 2014). The large particle size requires freshly made particles to be preserved in the gelling bath for a period of time. Although the droplet surface gels instantaneously upon entering the gelling solution, a longer time is needed for the cations to diffuse into the interior of the droplet depending on gel particle size (Gacesa, 1988).

Modified extrusion: A number of modified extrusion methods have been developed to overcome the weaknesses of the simple extrusion method and to produce micron size particles. These methods can be divided into three general categories (Ching et al., 2017):

(1) Jet break up extrusion: In this method, a laminar jet of polymer is formed by forcing the solution through a nozzle tip. The jet is then broken into discrete droplets by electrostatic atomization, vibrating nozzle or jetcutting.

(2) Spinning disk: Droplet formation is attained by the effect of centrifugal force acting on a flow of polymer

solution across a spinning disk or a rotating nozzle.

(3) Atomization: Droplets are formed by pumping air and polymer solution concurrently at high flow rates into a nozzle. Fine polymer droplets are formed when the air and polymer solution come in contact with each other.

Emulsification/gelation technique: Many researchers (Poncelet et al., 1992, 1995, 1999; Sultana et al., 2000; Chan et al., 2002; Lupo et al., 2014) have used this method. In this method alginate particles are formed in the oil phase. Alginate-active component solution is dispersed in an oil bath and homogenized to produce water-in-oil of emulsion. Therefore, when ionic induced gelation occurs, alginate forms the polymeric matrix trapping the active component inside. The preparation of alginate microspheres through *the emulsification/ gelation* technique may be carried out by means of *external or internal gelation.*

External gelation: In this method, an aqueous solution of alginate-active component is emulsified in oil phase. A $CaCl_2$ solution is then added to the medium to induce the gelation of droplets and promote separation of the formed micro- or nanoparticles (Poncelet et al, 1999, Mokhtari et al., 2017).

Emulsification/internal gelation: In this method an insoluble/partially soluble salt of calcium, usually $CaCO_3$, is already dispersed in the alginate solution droplets in oil emulsion (W/O). An acid is then added to the medium that must diffuse along the continuous phase into the droplets. Then their pH decreases and Ca^{+2} ions are slowly released within the ionic polysaccharide.

Emulsification/gelation method considered to be i fast, easily to scaled-up, and uses biocompatible materials. It also, allows to use soft conditions to make the particles (neutral pH, work at room temperature, use of non-toxic reagent. However, the limitation of this technique is the random droplet coalescence (Poncelet et al., 1999). The polydispersity of the particles cannot be easily controlled and very broad size distributions have been reported, see Figure 1.21, and the particles tend to coagulate into large masses before hardening properly (Poncelet et al., 1992, 1995; 1999; Lupo et al., 2014). The mean diameters of the microparticles ranged between 50 and 1000 microns although with low encapsulation efficiency (Poncelet et al, 1992). This may cause poor reproducibility in controlled release studies (Zhao et al., 2007).

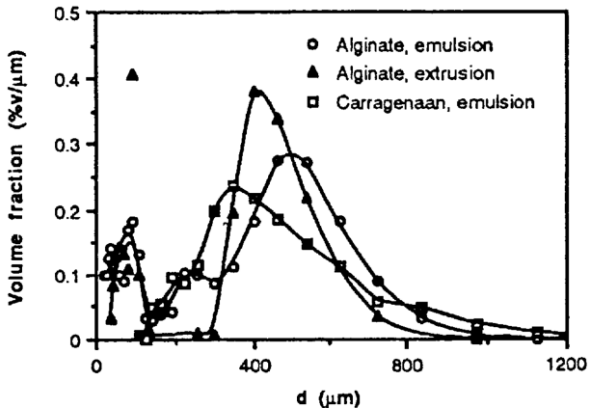


Figure 1.21. Size distribution of different beads using alginate emulsion, alginate extrusion and alginate carrageenan (Poncelet et al, 1992).

1.3.2.2. Hydrogel technical features

The functional features of an ideal hydrogel material can be listed as follows (Ahmed, 2015).

i. High absorption capacity (maximum equilibrium swelling) in saline.

ii. Desired rate of absorption (preferred particle size and porosity) depending on the application requirement.
iii. High absorbency under load (AUL).
iv. Low soluble content and residual monomer.
v. Low price.
vi. High strength and stability in the swelling environment and during the storage.
vii. Highest biodegradability without formation of toxic species following the degradation.
viii. PH-neutrality after swelling in water.
ix. Colourlessness, odorlessness, and absolute non-toxic.
x. Photo stability.
xi. Re-wetting capability (if required) the hydrogel has to be able to give back the imbibed solution or to maintain it depending on the application requirement (e.g., in agricultural or hygienic applications).

Obviously, it is impossible that a hydrogel sample would simultaneously fulfil all the above-mentioned required features. In fact, the synthetic components for achieving the maximum level of some of these features will lead to inefficiency of the rest. Therefore, in practice, the production reaction variables must be optimised such that an appropriate balance between the properties is achieved. For example, a hygienic products of hydrogels must possess the highest absorption rate, the lowest re-wetting, and the lowest residual monomer, and the hydrogels used in drug delivery must be porous and response to either pH or temperature.

1.3.2.3. Factors affecting Hydrogel features

Dimensions and morphology

The dimensions of hydrogels can also vary widely, ranging from nanometres to centimetres in width. They are also relatively deformable and readily conform to the shape of any space to which they are confined (Bhattarai et al., 2010). The size of the hydrogel particles affects the optical clarity, stability, physicochemical properties, and functional performance of delivery systems (Peppas et al., 2000). Precisely, particle size has a great impact in controlling both the stability and release of encapsulated materials from hydrogel particle delivery systems (Joye et al., 2014). The particles size can be controlled by employing the right

composition, fabrication method, and operating conditions, which allows producers to adapt them for specific applications.

Another factor that may influence the properties of hydrogel particles is their morphology. Sphere shaped particles are the commonly produced particles. However, hydrogel particles can be found in other shapes, for example, disks, fibres, oblongs, and ellipsoids. Furthermore, hydrogel particles may have other structure such as; homogeneous, core-shell, or dispersion, see Figure 1.22(A).

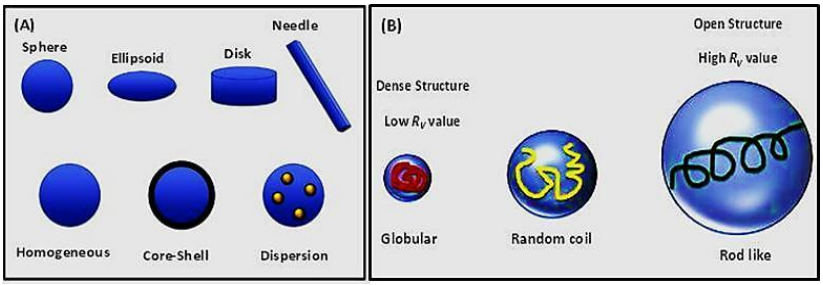


Figure 1.22. A) Different shapes and internal structures of hydrogel particles; B) Conformations of biopolymers in aqueous solutions (Zhang et al., 2015).

It is also worth to mention that in many applications, especially in drug delivery system; spherical calcium alginate beads with uniform size are desirable due to their well-defined geometry that allows a reproducible and controllable drug release profile (Voo et al., 2016).

Internal structure

The internal structure of hydrogel particles also plays an important role in determining their retention, protection, and release characteristics. Many physicochemical and structural parameters are typically used to define the internal structure of hydrogels, e.g., effective volume fraction, pore size, and cross-linking (Zhang et al., 2015).

❖ Effective volume fraction

As mentioned earlier that hydrogel particles consist of a network of aggregated biopolymer molecules with water molecules trapped between them. The fraction of a hydrogel particle that is actually occupied by the biopolymer chains has pronounced role on their encapsulation properties. Normally, as the concentration of the biopolymer inside the particle rises the rate of release of an entrapped material falls because it is more difficult for it to diffuse through the polymer

network. The effective volume fraction occupied by the biopolymer molecules Φ_B in a hydrogel particle is given by:

$$\Phi_B = {}^{V_B}/_{V_P} = {}^{1}/_{R_V} \quad (1.1)$$

Where V_B and V_P are the volumes of the biopolymer chains and the hydrogel particle, respectively. Equation (1.1) shows that the effective volume fraction is the inverse of the biopolymer volume ratio R_V, which is often used to describe the structural and physicochemical characteristics of individual biopolymer molecules in solution, Figure 1.22(B), as well as hydrogel particles. The R_V value increases as the amount of solvent molecules (usually water) enclosed by the biopolymer molecule or hydrogel particle increases (McClements, 2000). Thus, a swollen hydrogel particle with an open structure trapping a lot of water has a high R_V value, whereas a shrunken hydrogel particle with a dense, compact structure will have a low R_V value. The effective volume fraction of biopolymer chains contained by a hydrogel particle depends on many factors such as the initial concentration of the biopolymer, molecular weight, branching, charge density, flexibility, configuration, cross-link type, and interactions.

- **Pore size**

Pore size is another important property that influences the mechanical strength and encapsulation properties of hydrogel particles, see Figure 1.23. The pore size of hydrogel particles can often be tailored to achieve controlled or triggered a release of encapsulated bioactive, e.g., by changing polymer type or concentration, altering the type or degree of cross-linking, or applying external stimuli (e.g., pH, ionic strength, or temperature). High biopolymer concentrations and numerous strong cross-links within a hydrogel network promote the formation of small pores. The retention, protection, and release of encapsulated bioactive is closely related to the pore size of hydrogel particles (Zhang et al., 2015).

Hydrogels show a swelling behavior dependent on the external environment where polymer complexes can be broken, or the network can be swollen as a result of the changing external environment. They tend to show extreme changes in their swelling ratio as a result. Some of the factors that affect the swelling of hydrogels include pH, ionic strength, temperature and electromagnetic radiation (Hoare and Kohane, 2008; Peppas et al., 2000; Samchenko et al., 2011).

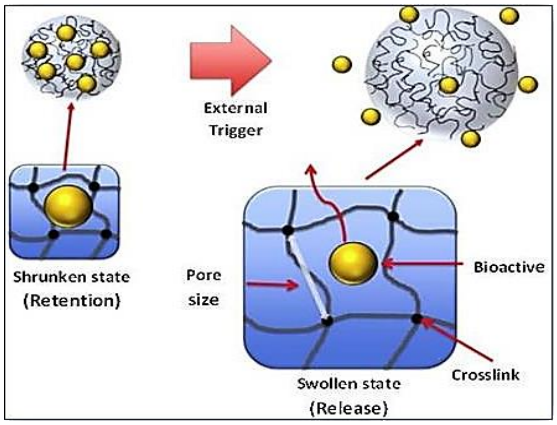


Figure 1.23. Schematic of pore size of hydrogel particles at swollen and shrunken state, respectively (Zhang et al., 2015).

❖ Cross-linking

The nature, strength, and number of the cross-links between the biopolymer molecules within a hydrogel particle are also important. The mechanical strength and encapsulation properties can be controlled by the degree of cross-linking of a hydrogel. The degree of cross-linking of a hydrogel is the number of cross-links per unit volume. The greater the cross-linking density, the stronger is the hydrogel and the slower is the release rate of any encapsulated materials. The nature of the cross-links determines how the hydrogel particle will respond to changes in environmental conditions. For example, hydrogen bonds dissociate when they are heated above a particular temperature, whereas electrostatic bonds may breakdown when the pH or ionic strength is changed. The degree of cross-linking can be controlled by increasing the concentration of biopolymers and cross-linking agents used to fabricate the hydrogel particles (Zhang et al., 2015). From literature, i.e., it has been shown that the ability of enzymes to penetrate into hydrogel particles decreases as the cross-linking is increased due to the increase of the biopolymer (alginate) or cross-linker (calcium) concentration (Li et al., 2011).

1.4. Concept of this work

❖ CPI Emulsification

From the brief literature review outlined above it can be concluded that the stability of the emulsion is directly associated with the drop size reduction achieved at the end of the emulsification process. In addition, the emulsification process is strongly affected by preparation methods that can lead to different drop size distribution, which is strictly related to the final emulsion stability. Processes that involve phase inversion can lead to the formation of finely dispersed drops in the continuous phase. Emulsions of finer drops can be achieved by different means; for example, by increasing the surfactant concentration, by decreasing the interfacial tension, by stirring in a more energetic way or for a longer period of time. These methods can be more or less effective depending on the case and how to combine the different effects to achieve the best product characteristics. It is also concluded that CPI may be induced by the addition of a dispersed phase or any other factors that can increase the rate of drop coalescence.

Despite the efforts that have been made, there are still uncertainties about the underlying processes by which finer emulsions are produced. Developments of emulsion properties such as emulsion conductivity and its effect on the emulsification process and the emulsion quality are still uncertain. Therefore, knowing when phase inversion takes place is essential for numerous industrial applications since the change in phase continuity will lead to a system with different properties and a complete explanation must be able to outline a wide range of experimental trends.

In this part of the work, we present a comprehensive practical study of the preparation of oil-in-water emulsions via catastrophic phase inversion and direct emulsification methods at different conditions. We investigate the conditions under which catastrophic phase inversion (CPI) can produce finer emulsions than direct emulsification. A model oil/water emulsion (cyclohexane/water) in the presence of non-ionic surfactants has been used. The changes in emulsion conductivities along the phase inversions were observed and the effects of different process and formulation parameters on the inversion time, the effective dispersed phase fraction, the internal phase fraction, the conductivity and the Sauter mean diameter $D_{3,2}$ of drops in the final emulsions were studied. Phase inversion process can be altered by the surfactant affinity toward phases and the

dynamic effects caused by variations in process parameters such as agitation intensity. In this study, both aspects are considered. The effective value of HLB of the surfactants is changed by either changing surfactant composition (the ratio between the oil soluble surfactant and the water-soluble surfactant) or by altering the emulsification temperature. The dynamic effects are explored by changing the mixing rate.

- **Multiple Emulsions**

From the brief literature review outlined above regarding multiple emulsions, it can be concluded that the encapsulation efficiency of multiple emulsions is directly associated with the drop size reduction achieved at the end of the process. The stability of multiple emulsions is strongly affected by preparation method that can lead to different drop size distribution. Various factors may affect encapsulation efficiency of multiple emulsions such as; mixing speed of the emulsification process, the type and the surfactant concentration, phase fraction s of the phases and the oil type and its viscosity. The effect of these parameters can be more or less effective depending on the case and how to combine them to achieve the best product characteristics.

Despite the efforts that have been made, there are still uncertainties about the underlying processes by which stable multiple emulsions are produced. Developments of emulsion properties such as release and its effect on the emulsion quality are still uncertain. Therefore, understanding release mechanism and how does it occur is essential for numerous applications in the industry. Since the changes in the emulsion conductivity will affect the release and in turn, this will affect the encapsulation efficiency, and a complete clarification must be able to outline a broad range of experimental trends.

In this chapter of the work, a practical study on the preparation of water-in-oil-in-water multiple emulsions is presented. Emulsions were prepared using two-step emulsification method. The effect of different parameters under which multiple emulsions can be produced was investigated. A model water-in-oil-in-water emulsion (water-in-paraffin-in-water) in the presence of non-ionic surfactants of different hydrophilicity has been used. The changes in emulsion conductivities were monitored, and the effects of different process and formulation parameters on the Sauter mean diameter $D_{3,2}$ of drops in the final emulsions were studied.

❖ **Hydrogel particles**

From the literature review defined above on the subject of hydrogel particles, it can be concluded that emulsification technique, currently used for producing alginate hydrogel particles, cannot produce uniform particles with narrow size distribution. Here a new modified emulsification method, based on mixing of emulsions of sodium alginate and calcium chloride, to produce macroporous hydrogel particles is introduced and investigated. By using a fine emulsion of $CaCl_2$ solution (using a homogeniser) instead of the solution itself, firstly the chance of coalescence between $CaCl_2$ droplets and sodium alginate drops in the oil phase increases. Secondly, it could improve the uniformity of particles in terms of size distribution. Effects of concentrations of sodium alginate and calcium chloride on the hydrogel shape and size and size distribution are investigated.

Chapter 2

MATERIALS AND METHODOLOGY

This chapter gives a detailed description of the materials used in this research, experimental set-up, and procedures followed for the preparation and characterisation of the emulsions.

2.1. Materials

All chemicals were obtained from Sigma-Aldrich, unless otherwise stated, and used without any further purification.

❖ CPI Emulsification

Cyclohexane (C_6H_{12}) was used as the oil phase, with purity ≥99%. Deionised water was used as the water phase. KCl (0.5% w/w based in the water phase) was dissolved in the water phase to enhance the electrical conductivity. The water-soluble grade non-ionic surfactant was Igepal (CO-720) of HLB of 14.2 (nonylphenylether length of 12) and the oil-soluble grade non-ionic surfactant was Igepal (CO-520) of HLB of 10 (nonylphenylether length of 5), see Figure 2.1. In all experiments, the total surfactant concentration was constant (5% w/w based on the water phase) which consisted of one of the above-mentioned surfactants or a combination of them.

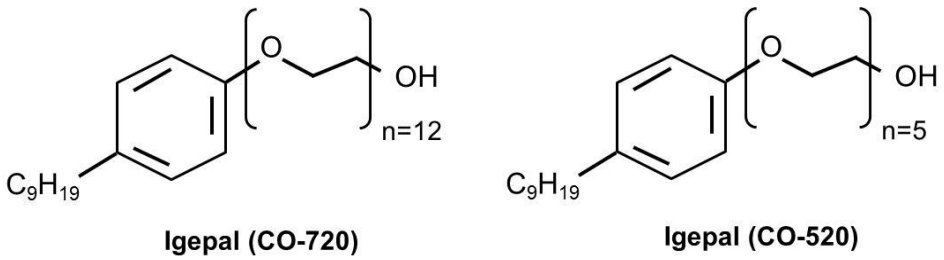


Figure 2.1. Chemical structure of Igepal (CO-720) and Igepal (CO-520).

❖ Multiple Emulsions

Heavy paraffin oil (C_nH_{2n+2}, n≈16-24) was used as the oil phase, and deionised water was used as the water phase. Tween 80 (Polyoxyethylene (20) sorbitan monooleate, $C_{64}H_{124}O_{26}$, HLB=15) and Span 80 (Sorbitan monooleate, $C_{24}H_{44}O_6$, HLB=4.3) used as hydrophilic and hydrophobic surfactant, respectively. NaCl was added to the internal water phase of the primary emulsion as the inner active agent (mostly at 0.1 M concentration). In a series of experiments, cyclohexane mixed with paraffin oil and used as the oil phase.

❖ Hydrogels

Sodium alginate $(C_6H_7NaO_6)_n$, see Figure 2.2, deionised water, vegetable oil (Bako), oil soluble surfactant, Span 20 (sorbitan laurate, $C_{18}H_{34}O_6$, HLB=8.6), and calcium chloride, $CaCl_2$, were used for preparation of hydrogels.

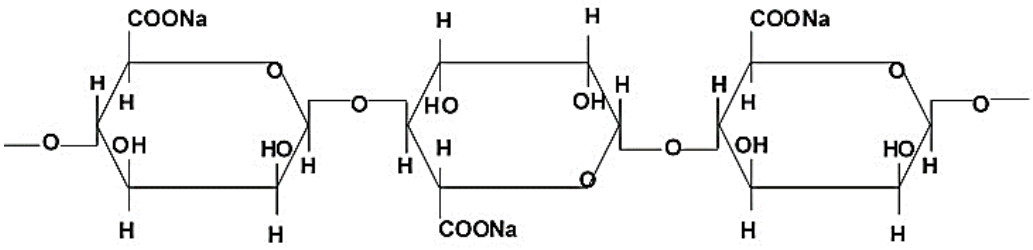


Figure 2.2. Chemical structure of sodium alginate.

2.2. Methodology

- **CPI Emulsification**

Experimental Setup

A jacketed glass reactor (250cm^3, 7cm in diameter) was employed for emulsification processes. The lid of the reactor made from PTFE (poly tetra fluoro ethylene), contained a 4 sealed neck with supported stainless steel frame to avoid the incorporation of air. A 4-blade turbine (4 cm in diameter) was used with blades equally spaced at 90° intervals. The agitator was connected to a digital variable speed motor (IKA euro star digital rotator) and placed at one-third of the height of the reactor from the bottom of the reactor.

Methods

Direct (D) and catastrophic phase inversion (CPI) methods were employed to prepare the O/W emulsions. ***Direct emulsification experiments*** were started first by adding the water phase containing KCl and the surfactant(s) mixture into the vessel, followed by the batch-wise addition of the oil phase via a glass column, under continuous stirring. ***Catastrophic phase inversion (CPI) emulsification experiments*** were started by adding the oil phase into the vessel, followed by the batch-wise addition of the water phase containing 0.5 wt% KCl, via a glass column, under continuous stirring. A couple of experiments were repeated to show the reliability of methodology and reproducibility of the results (see appendix A).

The effect of the surfactant location was considered. In some cases, the surfactant(s) mixture was dissolved in the oil phase, while in other cases it was dissolved in the water phase. The weight averages method was used to prepare the surfactants mixture with a predetermined HLB using the following formula:

$$HLBmix = f_A \times HLB_A + f_B \times HLB_B \quad (2.1)$$

where f_A and f_B are the weight fractions of surfactant *A* and *B*, respectively. The weighed surfactant(s) was then dissolved in the intended phase and left sealed for 22 hours before start of the emulsification.

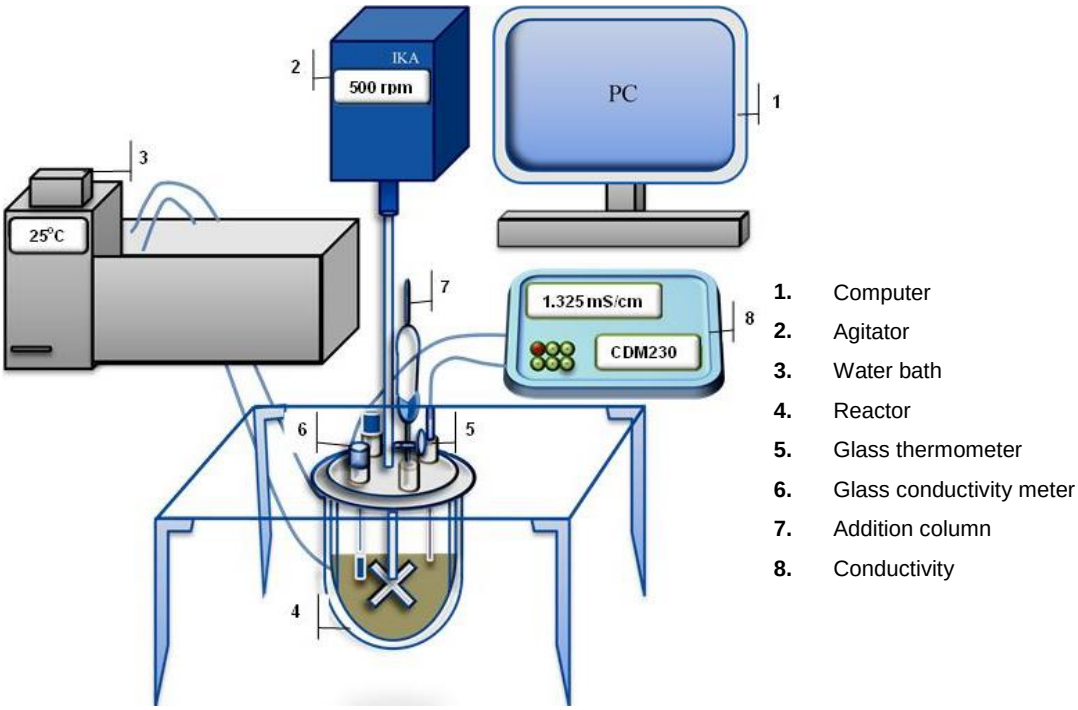


Figure 2.3. Experimental set-up.

❖ Multiple Emulsions

Experimental Setup

Multiple emulsions were prepared using a two-step emulsification method, as shown in Figure 2.4.

Method

The first step was to make the primary, W/O, emulsion. An aqueous solution of NaCl (0.1 M) was introduced into the oil phase containing a pre-determined amount of Span 80. The dispersion was homogenised for 2 min at a given stirring speed using Eurostar digital stirrer. In the second step, the primary emulsion was dispersed into a continuous water phase containing a pre-determined amount of Tween 80. The secondary emulsification was carried out using the same stirrer for 2 min at a given stirring speed. All experiments were conducted at room temperature (20°C), unless otherwise stated.

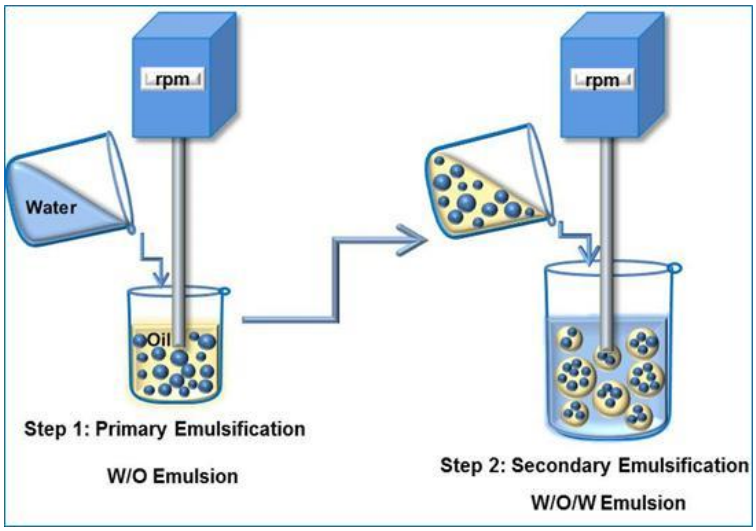


Figure 2.4. Schematic of two-step emulsification method for making multiple emulsions.

❖ Hydrogels

Experimental Setup

A series of preliminary experiments were conducted to synthesise hydrogel particles using a proposed two-step method based on inclusion. The jacketed glass reactor and the homogeniser were employed to prepare the W/O emulsions of and $CaCl_2$, respectively. The mixing of the two W/O emulsions were carried out in the jacketed glass reactor.

Method

A two-step method was proposed. In the first step two normal W/O emulsions were prepared. The oil phase was prepared by dissolving pre-determined amount of Span 20 in the oil (vegetable oil). For the NaAlg emulsion, a known amount of this salt was dissolved in water and the solution was introduced to the oil phase in the jacketed glass reactor and under continuous stirring (500 rpm) using the Eurostar digital stirrer. The $CaCl_2$ emulsion was prepared in a small beaker by introducing pre-prepared $CaCl_2$ solution to the oil phase while stirring using the homogeniser (3200 rpm) for 2 minutes. In the second step, the $CaCl_2$ emulsion was added to the NaAlg emulsion in the reactor at stirring speed of 500 rpm and stirred for 2 hours. Then the sample was filtered to separate the formed hydrogel particles. The obtained particles washed with water several times to remove the excess of the oil. Finally, the particles were dried in the oven at 50^{o}C for at least 8 hours. All experiments were conducted at 25^{o}C.

2.3. Characterisation

❖ CPI Emulsification

i. Phase inversion point

Inversion point was identified by monitoring the changes in the electrical conductivity of the emulsion. A digital conductivity meter, equipped with an analytical radiometer (SA, CDM230) (see Figure 2.5), was connected to the computer to monitor and record the emulsion conductivity during the emulsification process. The conductivity of the initial W/O emulsion continuously, but gradually, increased with time due to inclusion until a sudden jump in conductivity occurred when the W/O emulsion inverted to an O/W emulsion. In all experiments the aqueous phase contained KCl (0.5% w/w) to enhance the conductivity.

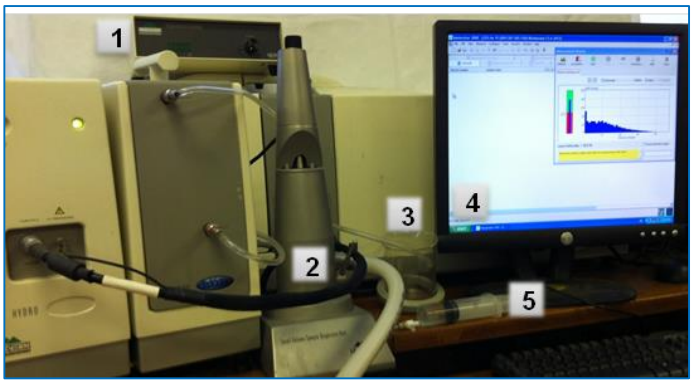
Figure 2.5. Conductivity meter (SA, CDM230).

ii. Drop size

The final Drop size of O/W emulsions was measured using a laser diffraction method (Malvern Master Sizer, UK), see Figure 2.6. Samples of a few drops were taken from the same position between the impeller and the wall of the reactor and placed in the small cell of the laser diffraction, which contained deionised water. Table 2.1 shows the optical properties for our system that used during the measurements.

Table 2.1. Optical properties used to measure O/W drop size using mastersizer.

Particle name	Cyclohexane oil
Particle refractive index	1.4262
Particle absorption index	0.001
Dispersant name	Water
Dispersant refractive index	1.33
Accessory name	Hydro 2000 µP



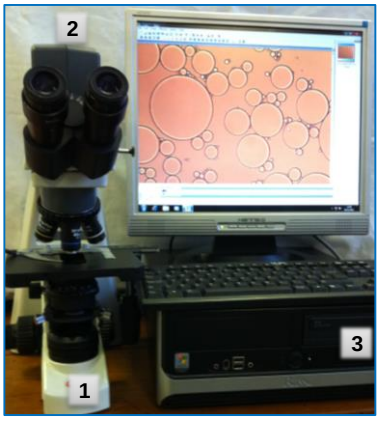
1. Dispersion unit controller
2. Unit cell
3. Beaker for waste
4. Computer
5. Injector

Figure 2.6. Laser Diffraction Particle Sizer System.

The measurement carried out twice; once at inversion time and once one hour after inversion time for all experiments.

iii. Drop morphology

Drop morphology was monitored using Motic optical microscope equipped with a digital camera (see Figure 2.7). For sample preparation, a few drops of the emulsion was diluted with distilled water, placed on the microscope glass slide and quickly covered with the cover slip before pictures were taken.



1. Optical microscope
2. Digital camera
3. Computer

Figure 2.7. Optical microscope.

❖ Multiple Emulsions

i. Drop size

Microscopic images of the primary W/O samples were used to estimate their drop size via the Image Pro Plus7.0 analysis software. This software measured the diameter of the drops. Approximately 200 drops were measured for each sample, and the mean Sauter diameter ($D_{3,2}$) was calculated. The drop size of the freshly-prepared multiple W/O/W emulsions were measured using the laser diffraction

particle sizer (Master sizer 2000, Malvern Instruments). Samples were first diluted with deionised water to prevent multiple scattering effects. They were then circulated through the small dispersion unit (the Hydro SM unit). Table 2.2 shows the optical properties for our system that used during the measurements.

Table 2.2. Optical properties used to measure W/O/W drop size using mastersizer.

Particle name	Paraffin oil
Particle refractive index	1.468
Particle absorption index	0.001
Dispersant name	Water
Dispersant refractive index	1.33
Accessory name	Hydro 2000 µP (A)

ii. Viscosity

The viscosity of multiple emulsions measured immediately upon their preparation at room temperature. The measurements carried out using Gimini 200 viscometer. Shear rate was ranging between 0.1 S^{-1} to 1000 S^{-1} and a gap size of 150 mm.

Figure 2.8. Viscometer (GIMINI 200).

iii. Drop morphology

The Motic optical microscope was used to observe the morphologies of the primary W/O emulsions as well as the multiple W/O/W emulsions drops. A few drops of the emulsion was placed on a slide and covered with a cover slip prior to analysis. Images were taken immediately after sample preparation.

- **Hydrogels**

The Motic optical microscope was used to observe and follow the formation of hydrogel particles and to measure their size. While the scanning electron

microscopy, SEM, (see Figure 2.9) was employed to investigate the morphology of the dry hydrogels. Prior to analysis, particles were coated with gold with approximately 5 nm thickness.

Figure 2.9. Scanning electron microscope (SEM).

Chapter 3

RESULTS AND DISCUSSION

This chapter covers all experimental results obtained throughout this work. They are also thoroughly analysed and discussed.

3.1. Phase Inversion Emulsification

In catastrophic phase inversion (CPI) emulsification the rate of inclusion, phase inversion, and drop size in inverted emulsion all are affected by surfactant affinity towards phases as well as variations in other process parameters. In this study, many process and formulation aspects have been studied, including water phase fraction (f_w), HLB of the surfactant(s), location of the surfactant(s), stirring speed and temperature of the system.

Normal O/W emulsions were produced using two techniques; (i) direct emulsification, and (ii) catastrophic phase inversion (CPI) emulsification via inversion of an abnormal W/O emulsion. Cyclohexane was used as the oil phase and two surfactant; one hydrophilic (Igepal 720) and one lipophilic (Igepal 520), were used individually or in a mixture to alter the HLB. Table 3.1 shows the different formulations used in this research. The experimental set up and procedures are presented in details in Chapter 2.

Table 3.1. Different formulations of CPI and direct emulsifications.

Surfactant location	f_w (%)	HLB	T(°C)	Stirring speed (rpm)
SO	0.1-0.5	14.2	25	500
SW	0.2-0.5	14.2	25	500
SO	0.1-0.5	12	25	500
SO	0.25	11-14.2	25	500
SO	0.25	13	25	350-700
SW	0.25	13	25	350-700
SO	0.25	14.2	25	350- 700
SW	0.25	14.2	25	350- 700
SO	0.25	11	25- 65	500
SO	0.25	13	25- 65	500
SO	0.25	14.2	25- 65	500

[S] = 5 wt% based on water.
[KCl] = 0.5 wt% based on water.
SO: Surfactant in oil.
SW: Surfactant in water.

In the following subsections first inclusion as the mechanism of CPI is discussed, and then the effects of the following parameters :

(i) Water phase fraction (f_w)

(ii) HLB of the surfactant(s)

(iii) Stirring speed

(iv) Temperature

on the rate of inclusion, inversion time (or inversion point), and final drop size of the inverted O/W emulsion are discussed. Also the results are compared with those from direct emulsification method to identify the conditions for which the CPI method produces finer emulsions.

3.1.1. Inclusion; The mechanism of CPI

O/W emulsions via catastrophic phase inversion were prepared by adding the water phase to the oil phase. Figure 3.1 shows typical conductivity-time variations in CPI emulsification. Under agitation, the inclusion of the continuous phase (oil) to dispersed phase (water drops) starts as the water drops contain a water-soluble surfactant and so they are susceptible of inclusion of the oil from continuous phase. Inclusion can be seen by (usually) gradual increase in the conductivity of the abnormal W/O emulsion. The rate and extent of the inclusion depends on the conditions, which will be discussed later. Generally, inclusion leads to formation of an abnormal multiple emulsion (O/W/O), and increase in effective dispersed phase fraction as water drop size increases with inclusion.

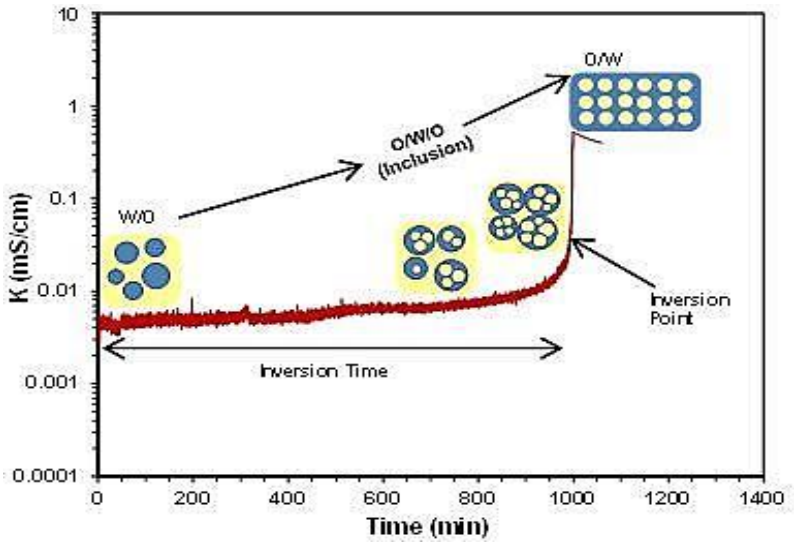


Figure 3.1. Inclusion and change in drop morphology from W/O to O/W emulsion in a typical CPI emulsification, (f_w = 0.1, [S] = 5 wt.%, HLB = 12 (SO), T = 25ºC, stirring speed = 500 rpm, [KCl] = 0.5 wt.%).

At a critical point (i.e., phase inversion point) the water drops rupture and the internal oil droplets are released when a normal O/W emulsion is formed. The conductivity of the system increases dramatically at this point which indicates the continuous phase has changed from oil to water. The phase inversion point for a typical experiment has been shown in Figure 3.1.

3.1.2. Experimental results and discussion

3.1.2.1. Water phase fraction (f_w)

Two sets of experiments were conducted to study the effects of water phase fraction (f_w) on the rate of inclusion, inversion time, and average oil drop size, $D_{3,2}$. In one set the effect of employing a single surfactant (CO-720, HLB=14) was studied while in the other set a mixture of surfactants (CO-720 and CO-520, HLB=12) were used. Also the effect of the location of surfactants (water phase; SW, and oil phase; SO) were studied.

Single surfactant (HLB=14)

The variation of the conductivity with time during catastrophic phase inversion (CPI), for an abnormal emulsion containing 5 wt.% single surfactant for different f_w, is presented in Figure 3.2(A) for SO when the surfactant was dissolved in the oil phase, and Figure 3.2(B) for SW, when the surfactant was dissolved in the water phase.

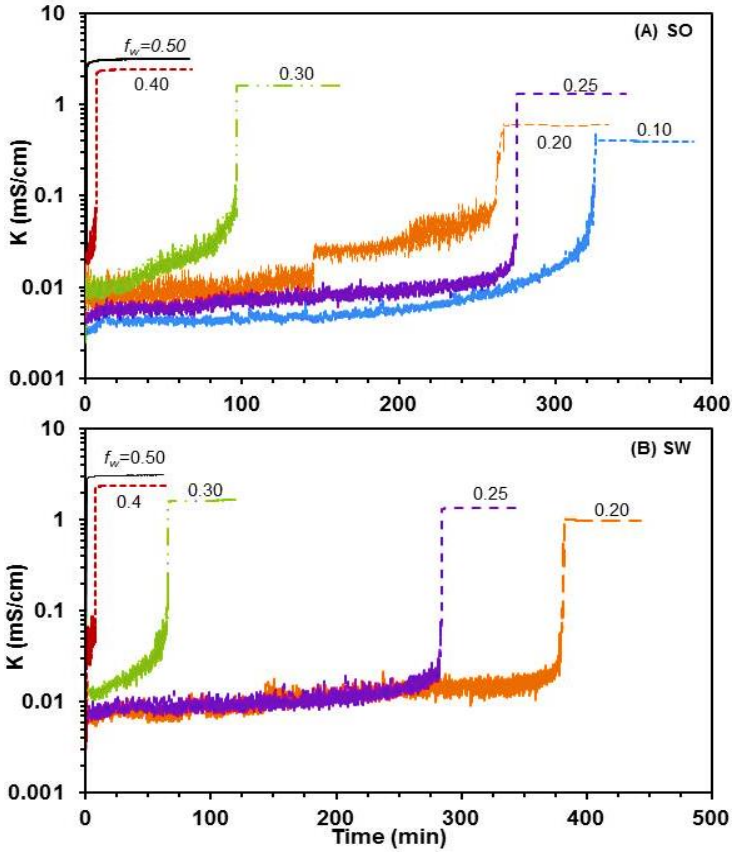


Figure 3.2. Time evolution versus conductivity at different f_w for CPI; A) SO, B) SW (HLB = 14, T = 25ºC, stirring speed = 500 rpm, [S] = 5 wt.%, [KCl] = 0.5 wt.%).

The results show that the rate of inclusion is faster as f_w increases.

Phase inversion time refers to the time needed for sufficient droplets from the continuous phase to be encapsulated into the dispersed drops for the effective dispersed fraction volume to be reached. In the case of higher water volume fraction, the inclusion happens very fast and so the inversion time is very short. At lower values of f_w inclusion is gradually developed before inversion point is reached. From the conductivity measurements illustrated in Figure 3.2, it is also clear that the conductivity of the normal emulsions increases with f_w, as expected (higher water content of normal emulsion at higher f_w).

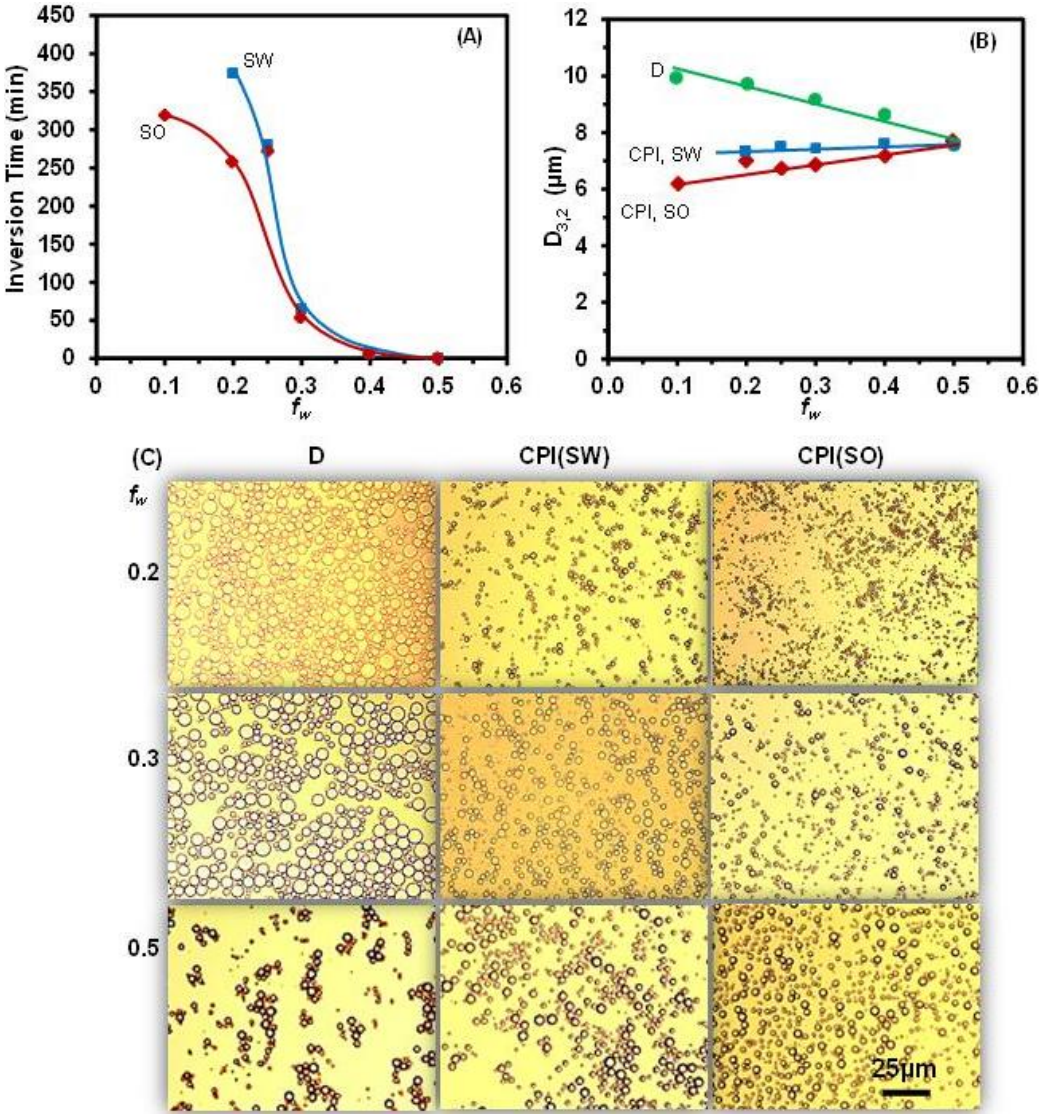


Figure 3.3. A) Inversion time, and B) $D_{3,2}$versus f_w for CPI (HLB = 14, T = 25ºC, stirring speed = 500 rpm, [S] = 5 wt.%, [KCl] = 0.5 wt.%), C) Micrographs of the droplets.

Figure 3.3(A) shows the effect of the water volume fraction f_w on phase inversion time. It is clear that phase inversion time decreases when the water fraction

increases for both SO and SW systems. Phase inversion time at f_w= 0.50 is close to zero indicating an immediate inversion. This could be due to the high rate of drop coalescence at higher f_w. Figure 3.3(B) compares the Sauter mean diameter $D_{3,2}$ of the oil droplets in the emulsions obtained by CPI (SO, SW) and D methods in terms of water volume fractions (f_w). Measurements were taken one hour after phase inversion. It is evident from Figure 3.3(B) and Figure 3.3(C) that both the D and the CPI method produce the same drop size at the higher f_w of 0.50. The comparison also shows that at f_w lower than 0.50, the direct method (D) produces bigger droplets than the CPI method regardless of the surfactant location.

The difference between the two methods at a given HLB also seems to widen with decreasing f_w. Also, as f_w decreased to 0.10, CPI (SO) produced significantly smaller droplets than CPI (SW) and it appeared to be more efficient. The reason for this could be due to the size of the internal oil droplets. Sajjadi et al. (2003a) reported that the increase in the size of a multiple drop with f_w, inclusion, due to drop deformation and drop coalescence, resulted in the formation of larger internal oil droplets.

It is believed that the formation of fine drops in CPI (SO) could be due to a lower dynamic interfacial tension caused by the diffusion-controlled surfactant transport from the oil phase to the continuous phase via the interface (Jahanzad et al., 2009). In the CPI (SO) method, when the surfactant is placed in the oil phase, the water-soluble surfactant has a longer chain length than the oil-soluble one and it diffuses toward the interface at a slower rate.

Furthermore, it is quite likely that some of the water-soluble surfactant is solubilised in the interior of the oil-soluble surfactant micelles in the oil phase, thereby delaying the diffusion. The slower diffusion of the water-soluble surfactant from the oil phase to the interface will cause the actual HLB of the emulsion at the water-oil interface to be smaller than the formulation of HLB and closer to the optimum HLB (10.5) (Jahanzad et al., 2009). This is also in agreement with previous findings by Lin et al. (1975); they found that when the surfactant dissolves in the oil phase, CPI produces a finer O/W emulsion than the emulsion, with the same composition, prepared by initially placing the surfactant in the water phase.

In order to compare the kinetic of the inclusion from the external continuous phase, the development of the dispersed phase fraction of the internal phase is

presented as a function of dimensionless time or relative time. Relative time is defined as time divided by the inversion time.

Bruggeman's formula was applied to predict the effective dispersed phase fraction f_d (Bruggeman, 1935; Bruggeman, 1936).

$$1 - f_d = \frac{K - K_d}{K_C - K_d}\left(\frac{K_C}{K}\right)^{1/3} \quad (3.1)$$

where K is the conductivity of emulsion, K_C is the conductivity of the continuous oil phase, K_d is the conductivity of the dispersed phase (water containing 0.5 wt% KCl), measured at 7.95 mS/cm, and f_d is the effective dispersed phase fraction. The conductivity of the oil phase could not directly be measured (the readings was too low or zero). In order to calculate the effective dispersed phase fraction (f_d), K_C value has been estimated using equation 3.1 with assuming $f_d = f_w$ (i.e., at the start of the emulsification process). The average value of K_C was estimated as 0.0023 mS/cm. The internal dispersed phase fraction (Ø) was calculated using the following equation:

$$\text{Ø} = \frac{f_{di}}{f_d} = \frac{f_d - f_w}{f_d} = 1 - \frac{f_w}{f_d} \quad (3.2)$$

Where f_{di} is the (average) volume fraction of oil droplets in water drops. Figure 3.4 shows multiple droplets of water in the abnormal W/O emulsion when the internal oil droplets are included in the water drops by inclusion.

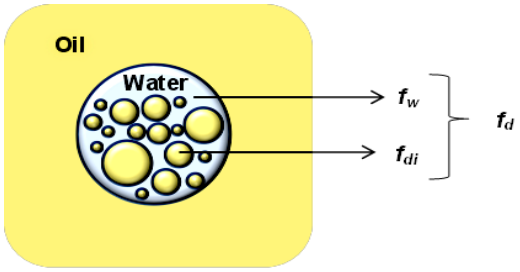


Figure 3.4. Schematic presentation of the internal dispersed phase (f_{di}), water volume fraction (f_w) and the effective dispersed phase fraction (f_d) in an abnormal O/W/O emulsion.

Here a sample calculation is demonstrated. For experiment conditions of HLB=13 (SW), and $f_w = 0.25$, the conductivity of the abnormal W/O emulsion at a give time was measured as $K = 0.01134$ mS/cm.

$K_d = 7.95$ mS/cm (measured)

$K_C = 0.0023$ mS/cm (calculated estimated value)

$$f_d = 1 - \frac{K - K_d}{K_C - K_d}\left(\frac{K_C}{K}\right)^{1/3}$$

$$f_d = 1 - \frac{0.01134 - 7.95}{0.0023 - 7.95}\left(\frac{0.0023}{0.01134}\right)^{1/3}$$

$$f_d = 0.413$$

$$\emptyset = 1 - \frac{0.25}{0.413}$$

$$\emptyset = 0.395$$

Figure 3.5(A and B) show the effective dispersed phase fraction versus relative time for SO and SW, respectively, while Figure 3.5(D and E) show the internal phase fraction versus relative time for SO and SW, respectively, at different f_w for CPI with HLB=14. These figures show that both the effective dispersed phase fraction and the internal phase fraction are countinously increasing with relative time until phase inversion occurs. This is due to inclusion of continuous phase inside dispersed phase and formation of multiple drops, which occurs due to the deformation of the dispersed drops, water drops in this case, by shear (Sajjadi et al., 2002a). In order to enhance the ability of the dispersed phase to include the continuous phase, it is necessary for an appropriate surfactant to be present in the dispersed phase (Salager et al., 1983).

Sajjadi et al. (2002a) argued that the deformation of the dispersed drops leads to a portion of the continuous phase to be included as droplets into the dispersed drops. This leads to a curvature favourable to the absorption of the surfactant present in the system, which in turn will pull the continuous phase inside the dispersed drops to form multiple drops, until it finally ruptures and releases the inner droplets. However, Klahn et al. (2002) suggested that, in addition to the inclusion mechanism, there is another process undertaken at the same time: escape mechanism. During this mechanism, inner droplets escape back into the continuous phase. It is then believed that when the inclusion rate overcomes the escape rate, multiple drops will grow in size as inclusion continues to involve more droplets of the continuous phase until a threshold in the effective dispersed phase fraction is reached and phase inversion to the opposite morphology takes place. Groeneweg et al. (1998) reported that it is possible that a steady state is reached at which the rate of inclusion is equal to the rate of escape. In this case,

inversion will not take place anymore. The results presented in Figure 3.5 are considered to be supportive evidence for the formation of multiple drops, and so inclusion, in this research.

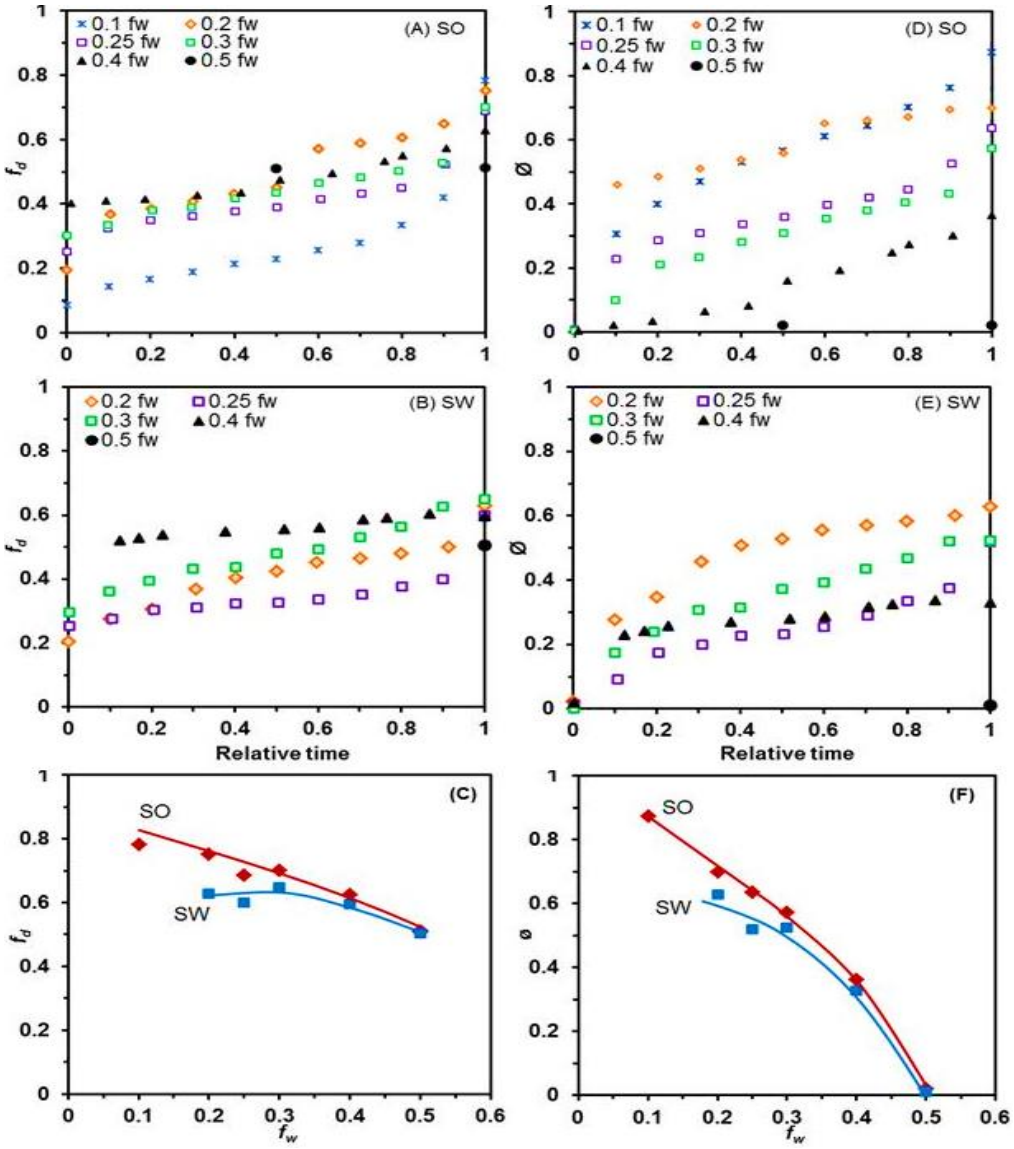


Figure 3.5. (A) and (B) The effective dispersed phase fraction for SO and SW, respectively. (D) and (E) The internal phase fraction for SO and SW, respectively versus relative time; (C) and (F) The effective dispersed phase fraction and the internal phase fraction versus f_w at inversion time, for CPI (HLB = 14, T = 25ºC, stirring speed = 500 rpm, [S] = 5 wt.%, [KCl] = 0.5 wt.%).

Figure 3.5(C) and (F) show the effect of f_w on the effective dispersed phase fraction (f_d) and the internal phase fraction (Ø) at inversion time. Both f_d and Ø decrease as the water volume fraction (f_w) increases. Interestingly, at higher f_w the internal phase fraction (Ø) is close to zero and thus phase inversion to the opposite morphology occurs immediately without inclusion of the continuous phase inside the dispersed phase. At lower f_w, both f_d and Ø are higher when the

surfactant is dissolved in the oil phase than when the surfactant is dissolved in the water phase for the reasons already discussed above.

Mixed surfactants (HLB=12)

In this set, the weight-average method was used to prepare the surfactant mixture with a predetermined HLB of 12 using equation 2.1. The surfactant mixture was then dissolved in the oil phase. Figure 3.6 shows the conductivity changes with time in CPI at different water fractions(f_w) using a mixed surfactant (HLB=12). Similar to Figure 3.2(A) for SO, the results show that conductivity for abnormal emulsions increases with time this is due to inclusion of the continuous phase inside the dispersed phase. Also the conductivity at inversion time inversion increases as f_w increases.

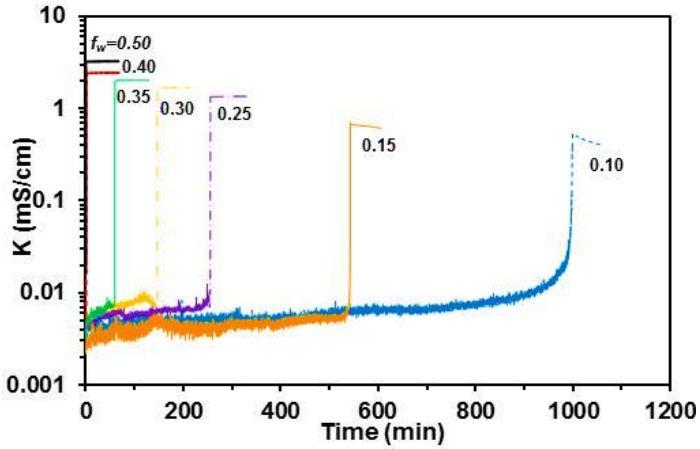


Figure 3.6. Time evolution versus conductivity at different f_w for CPI (SO) (HLB = 12, T = 25ºC, stirring speed = 500 rpm, [S] = 5 wt.%, [KCl] = 0.5 wt.%).

Figure 3.7(A) represents the inversion time for CPI (HLB=14 and HLB=12, both SO) showing both follow the same trend. As stated above, for higher f_w, the inversion time is shorter than that for lower f_w, which could be due to the higher rate of drop coalescence at higher f_w. However, inversion time for the mixed surfactant (HLB=12) is longer than the inversion time for a single surfactant (HLB=14) due to the presence of the hydrophilic surfactant. The longer chains of the hydrophilic surfactant (Igepal CO-720) will take longer to migrate from the oil phase to the water phase. Figure 3.7(B) compares the Sauter mean diameter $D_{3,2}$ for the CPI and D methods in terms of f_w using a mixed surfactant of HLB=12. For both methods, CPI and D, $D_{3,2}$ decreases with f_w. At lower f_w the difference in the size of the drops obtained from the two methods is more important than at higher f_w. However, CPI produces smaller drops than the direct

method. Comparing this figure with Figure 3.3(B) for CPI (HLB=14, SO), it can be seen that CPI produces finer droplets when mixed surfactants are used (HLB=12). This is in agreement with a previous work of Sajjadi and his co-workers. Their study showed that the drop size of the final emulsion decreases as the HLB of the surfactant decreases (Sajjadi et al., 2003a).

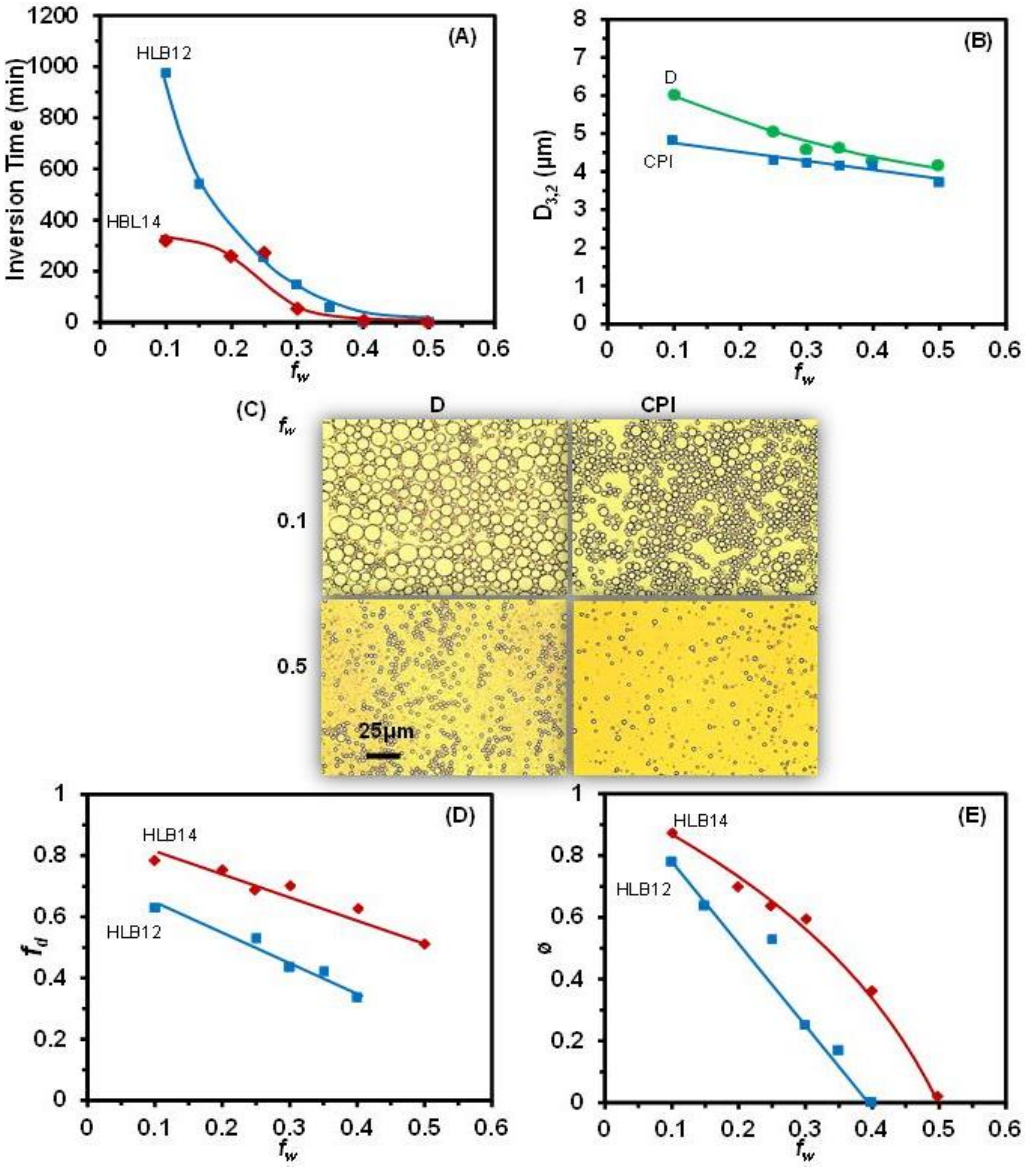


Figure 3.7. A) Inversion time, and B) $D_{3,2}$ for HLB = 12 versus f_w C) Corresponding micrographs for HLB = 12, (D and E) Effective dispersed phase fraction and internal phase fraction, respectively, versus f_w at inversion time for CPI (SO) (T = 25ºC, stirring speed = 500 rpm, [S] = 5 wt.%, [KCl] = 0.5 wt.%).

The study also found that the size of the internal droplets showed an appreciable decrease with decreasing HLB. As the surfactant HLB approached the optimum, the affinity of the surfactant mixture toward the oil phase was increased. This is

associated with a decrease in the interfacial tension, which favours more break-ups. Micrographs for the droplets obtained are shown in Figure 3.7(C).

The results obtained in Figure 3.7(D and E), show the effective dispersed phase fraction, f_d, and the internal phase fraction, Ø, versus f_w at inversion time, respectively. The results show that both f_d and Ø decrease as f_w increases, however, when high HLB is used, f_d and Ø are greater than when lower HLB is used. This means that the inclusion increases as the HLB of the surfactant increases as mentioned before.

3.1.2.2. Surfactant affinity (HLB)

In a new set of experiments different values of HLB (SO) at constant value of f_w=0.25 were employed. Different HLB values, ranging between 11 and 14, generated using a combination of the two surfactants (Igepal CO-720 and Igepal CO-520). Figure 3.8 shows the time evolution of conductivity during CPI emulsification for different HLBs at a constant volume fraction of water (f_w=0.25). The results show that the conductivity of the abnormal emulsion (O/W/O) at inversion time seems to increase as the HLB of the surfactant increases. Whereas the conductivity for the normal emulsion after inversion seems to fall in the same range for all experiments.

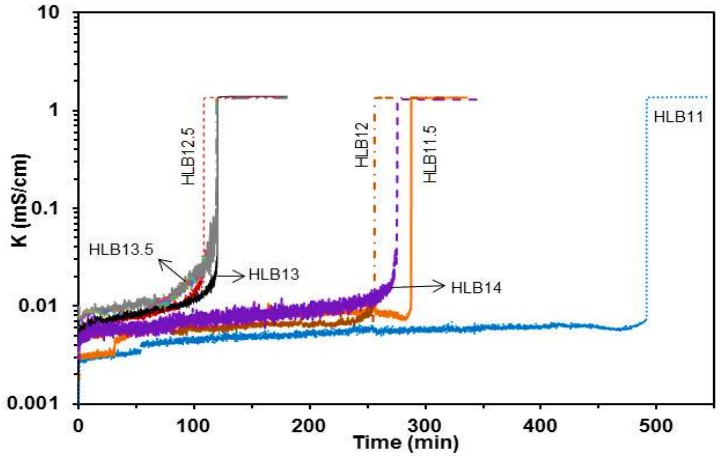


Figure 3.8. Time evolution versus conductivity at different HLB for CPI (SO), (f_w) = 0.25, T = 25ºC, stirring speed = 500 rpm, [S] = 5 wt.%, [KCl] = 0.5 wt.%).

The inversion time shows a minimum around HLB=13 as clearly shown in Figure 3.9(A). This is apparantly in agreement with the work of Rondón-González et al. Which found that the inversion time undergoes through a minimum for both mixed surfactants or single surfactant (Rondón-González et al., 2009). This is because

the inversion process passes through the formation of a multiple emulsion, they concluded that the inversion time depends on the capacity of the system to include the external phase as droplets and on the stability of the resulting inner emulsion. The resistance to coalescence of these inner water droplets in the multiple W/O/W system before inversion could be related to the stability of the normal W/O emulsion after inversion since the water drops after inversion mostly correspond to the inner water droplets before inversion. Rondón-González et al. monitored the stability for three W/O normal emulsions after inversion, prepared at different HLB values, the volume fraction of water separated as a function of time was measured after about five months. They found that the phase separation data match with the inversion time variation: as the system moves toward optimum formulation, the W/O emulsion first becomes more stable (the fraction of separated water decreases), then passes through a maximum stability, and finally becomes unstable as the formulation approaches the optimum . This result, strongly suggests that when inversion is induced by the continuous stirring of an abnormal W/O/W multiple emulsion, the inversion time is directly linked to the stability of the inner W/O emulsion. They also suggested that when two surfactants with highly different hydrophilicities are mixed the minimum inversion time appeared to be the consequence of the segregation of the two species between the two interfaces of the multiple emulsion.

However, They argued that this is not the case if only one surfactant is used. They reported that the system prepared with only one surfactant exhibits the same trend in inversion time as the system prepared with two surfactants; i.e., a minimum of inversion time is also observed. Certainly, the nonionic surfactant used, either Igepal CO-520 or Igepal CO-210, is not actually an isomerically pure substance, but a mixture of oligomers. They concluded that it is not the presence of two surfactants, eventually stabilising separately each interface, which explains the existence of a minimum of inversion time. It tends to indicate that the effect is due to the global formulation of the system, which, in the case of two surfactants, corresponds probably to mixed interfacial films formed rapidly at both interfaces, because of the low molecular weight of surfactant molecules. In our study same trend in inversion time is also observed; a minimum in inversion time was observed for HLB=12.5-13.5 when a mixture of surfactants used. Figure 3.9(B) compares the Sauter mean diameter $(\mathrm{D}_{3,2})$ of the final drops for the CPI and D methods in terms of HLB at f_w=0.25. $\mathrm{D}_{3,2}$ increases as the HLB of the surfactant

increases for both the D and CPI methods. However, the difference between the two methods is more significant at higher HLB. The results also show that CPI produces finer drops than the D method. Micrographs for the droplets obtained are shown in Figure 3.9(C).

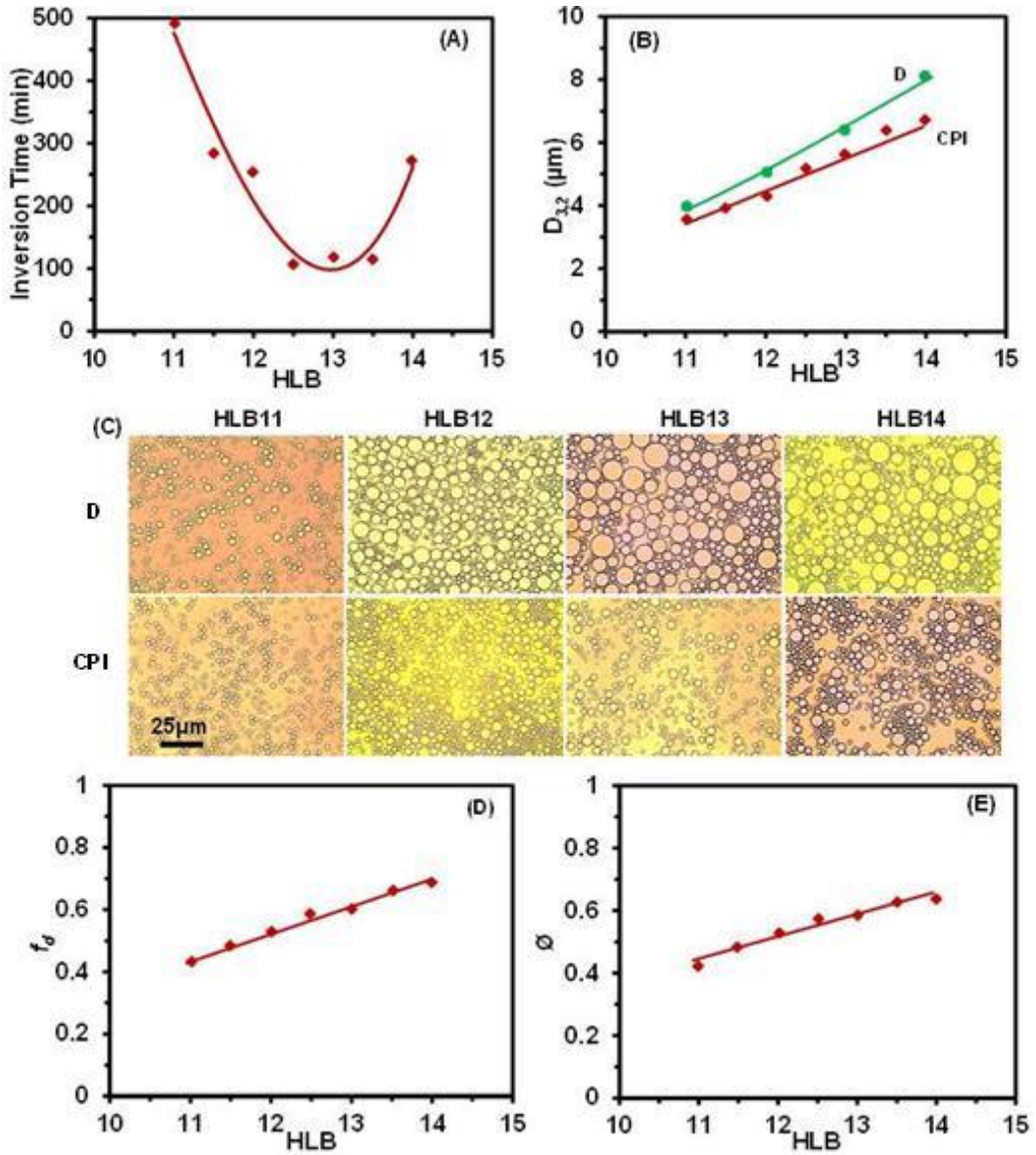


Figure 3.9. A) Inversion time, and B) $D_{3,2}$ versus HLB C) Corresponding micrographs, (D and E) Effective dispersed phase fraction and internal phase fraction respectively, versus HLB at inversion time for CPI (SO) (f_w) = 0.25, T = 25ºC, stirring speed = 500 rpm, [S] = 5 wt.%, [KCl] = 0.5 wt.%).

As mentioned above using mixed surfactants leads to reduce the interfacial tention between the oil and the water drops which inturn leads to produce fine emulsions, which probably means they are more stable at lower HLB. The higher HLB increased the affinity of the dispersed phase to entrain the larger amount of oil from the continuous phase (i.e., enhanced inclusion), forming multiple drops.

Drop coalescence mechanism was responsible for inclusion at a higher HLB, but drop deformation mechanism was responsible for inclusion at a lower HLB.

Figure 3.9(D) and (E) show the effective dispersed phase fraction and the internal phase fraction versus HLB at inversion time, respectively, for CPI (SO) at f_w=0.25. These two figures show that both effective dispersed phase fraction and internal phase fraction continuously increase with HLB. This is due to the increase in the inclusion of the continuous phase inside the dispersed phase.

3.1.2.3. Stirring speed during emulsification

Single surfactant (HLB=14)

Two sets of experiments were carried out to study the effect of stirring speed on the CPI and D methods. A single surfactant of HLB14 was used and the surfactant location considered.

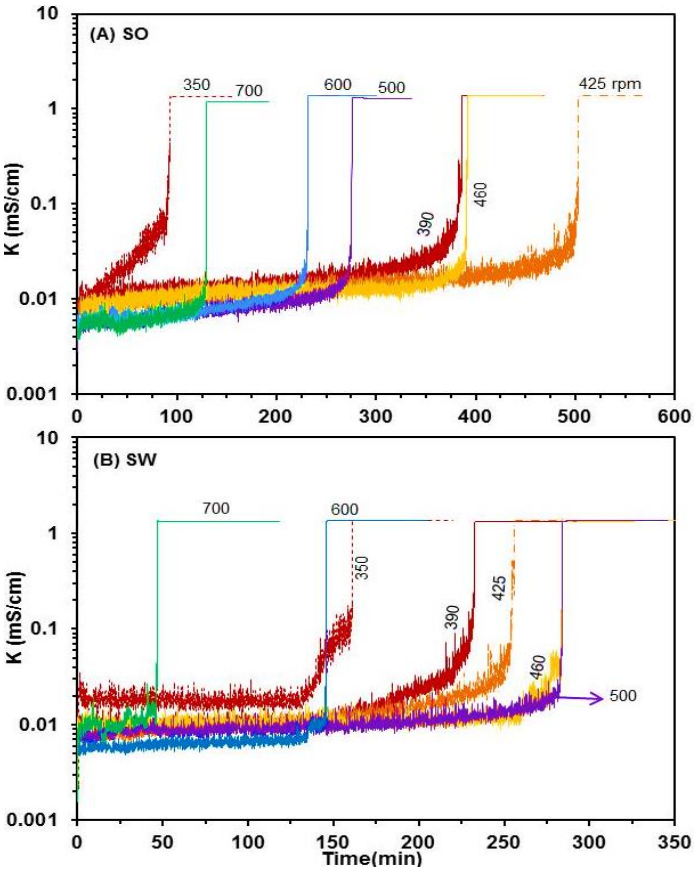


Figure 3.10. Time evolution versus conductivity at different stirring speeds for CPI; A) SO, B) SW (HLB = 14, [S] = 5.0 wt.%, f_w) = 0.25, T = 25ºC, [KCl] = 0.5 wt.%).

Figure 3.10(A and B) shows the conductivity changes with time in CPI (SO) and CPI(SW), respectively at different stirring speeds using a single surfactant (HLB14). The results of these two figures show that the conductivity at inversion

time decreases as the stirring speed increases; the conductivity at inversion time for SW is higher than conductivity for SO, while the conductivity after phase inversion is the same for both systems: SO and SW. Another point that can be drawn from these figure is that the rate of inclusion is faster as stirring speed decreases and it is greater when the surfactant is dissolved in the oil phase. This is due to the large size of multiple drops.

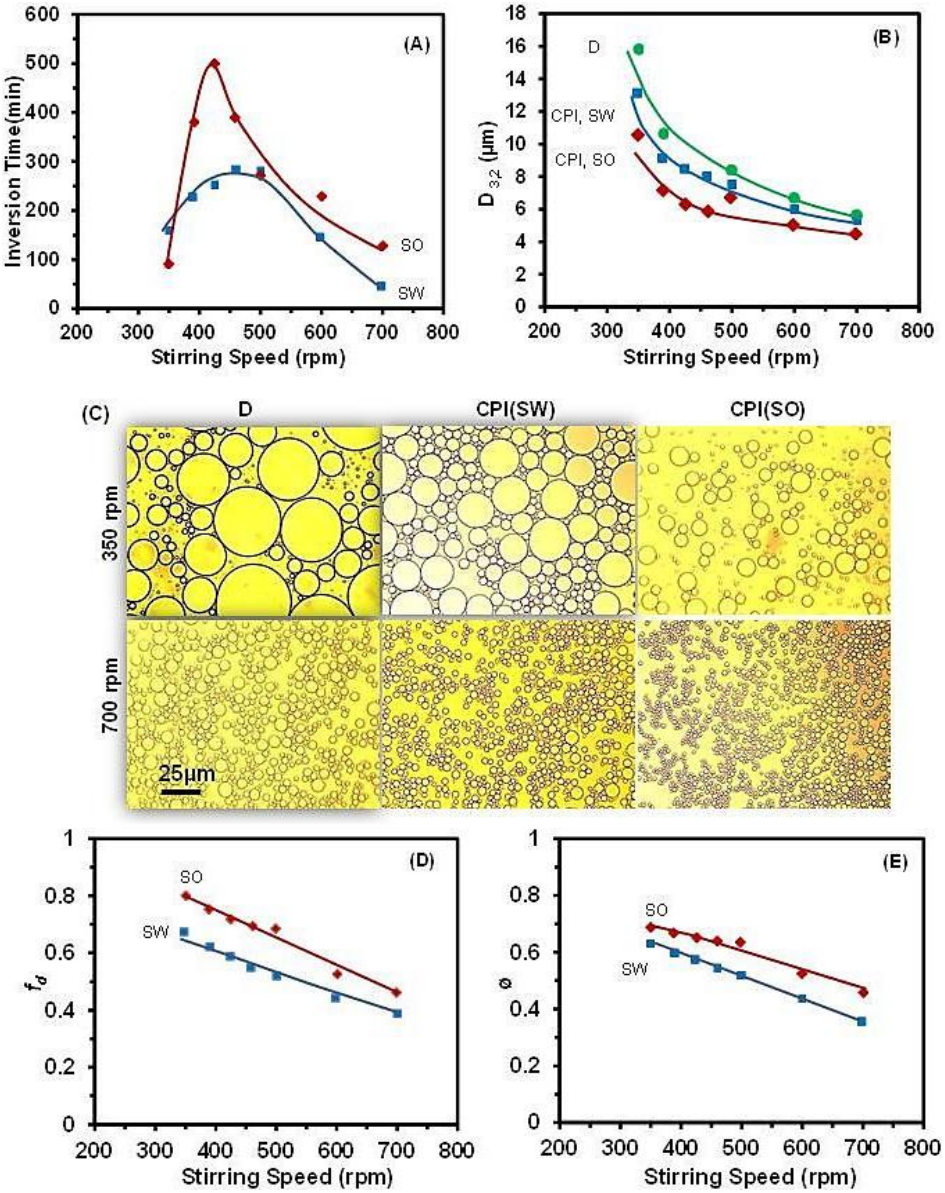


Figure 3.11. A) Inversion time; B) $D_{3,2}$versus stirring speed for CPI, C) Corresponding micrographs(D and E) Effective dispersed phase fraction and internal phase fraction versus stirring speed at inversion time, (HLB = 14, f_w) = 0.25, T = 25ºC, [S] = 5.0 wt.%, [KCl] = 0.5 wt.%).

Figure 3.11(A) shows the inversion time for CPI (SO, SW) using different stirring speeds (350-700 rpm). In this study, 350 rpm is the minimum mixing energy required to produce a homogeneous system. The results show that inversion time

initially increases with stirring speed, but later decreases, showing a maximum delay in the intermediate range. This is apparantly in agreement with what Mira et al. found for their CPI experiments at HLB=7.1 (Mira et al., 2003). They suggested that in an intermediate stirring speed zone two opposing effects should cancel each other off to cause the maximum delay in inversion. They explained that a low stirring regime is associated with the formation of large drops, i.e. low interfacial curvature, that can easily inverse to the opposite morphology. Thus, the inclusion of droplets inside the drops increases, leading to the formation of multiple emulsions. They argued that a high stirring regime forms very small droplets, which exhibits a high interfacial curvature and a large surface area, causing quicker surfactant adsorption (by convection rather than diffusion) at the interface which means normal morphology is favoured. Moreover, high turbulence increases the drop collision rate and coalescence into a continuous phase. They concluded that near to the intermediate stirring speed the drops are neither large nor very small, they are just small enough to exhibit a curvature that is difficult to reverse and the mixing is not energetic enough to adsorb the surfactant at the interface. As a result, the drops may not be packed by droplets, less coalescence occurs between the drops, and hence a maximum delay before inversion is exhibited in this region.

Here a maximum in inversion time was observed for HLB=14 only and when a mixture of surfactants used (see next section) there was no sign of a maximum in inversion time. This reveals that the maximum in inversion time is related to the high HLB of the surfactant used. We think that the long chains of non-ionic surfactant in Igepal CO-720 is responsible for this behaviour as it adds to the difficulty of diffusion of surfactant to the surface of drops at medium stirring speeds where none of the factors mentioned above (low curvature and surfactant adsorption by convection) is dominant. In another word, in intermediate stirring speeds the inversion is controlled by rate of diffusion of surfactant (diffusion-controlled inversion) and when only a long-chain surfactant is used inversion is delayed by slow diffusion of these chains (Jahanzad et al., 2009). Mira et al., 2003 performed only three experiments in high, intermediate and low speeds and discussed the behaviour of the system based on their results. It seems that their reasoning is not generic and the results needed to be verified by more experiments in different stirring zones. Yet, the results in Figure 3.11(A) show that the inversion time for SO is longer than the inversion time for SW; this is

probably because the surfactant dissolved in the oil phase takes more time to migrate from the oil phase to the water phase (Lin et al., 1975). Figure 3.11(B) shows the effect of stirring speed on average drop size $D_{3,2}$; as stirring speed increases, the average drop size decreases. At low stirring speed, the difference in drop size between the two methods is high, but the difference diminishes at high stirring speed. An increase in stirring energy generally leads to a decrease in drop size because it affects the drop breaking rate more than the coalescence rate. Thus, an increase in stirring energy is expected to widen the region where the decreasing tension produces smaller drops (Salager et al., 2002). This figure also reveals that at low stirring speeds the surfactant location has a significant influence on the average drop size of the normal emulsion. The emulsion prepared by initially placing the surfactant in the oil phase has finer droplets (more stable emulsion) than the emulsion prepared by placing the surfactant in the aqueous phase as has been discussed above and in agreement with Lin et al. (1975).

Micrographs of the final emulsions produced at different stirring speeds are shown in Figure 3.11(C) and clearly show the same trend. They also show that the direct method produces bigger drops than CPI method. The results are similar to the results obtained using Mastersizer laser diffraction. Figure 3.11(D) and (E) show the effect of stirring speed on f_d and Ø at inversion time. The results show that both f_d and Ø decrease as stirring speed increases. Another point that can be drawn from these figures is that inclusion increases as stirring speed decreases and it is greater when the surfactant is dissolved in the oil phase. This is due to the large size of multiple drops. At a lower stirrig speed, the effect of surfactant location on f_d is very high, while at higher values there is a small effect for all systems prepared at the same f_w. However, the effect of surfactant location on Ø at lower values is less than that at higher stirring speed as expected.

Mixed surfactants (HLB=13)

Two sets of experiments were carried out to study the effect of stirring speed on the CPI and D methods using a mixture of two non-ionic surfactants (Igepal CO-720, Igepal CO-520) at HLB of 13. The surfactant location was considered.

Figure 3.12(A) shows the time evolution of conductivity for CPI for SO. The results show that the conductivity at inversion time decreases as stirring speed

increases. It is also clear that inversion time decreases with increasing stirring speed for both SO and SW (also see Figure 3.13(A)) and the surfactant location is not very important when a mixture of surfactants is used to stabilise the emulsion. As mentioned above, comparing this figure with Figure 3.10(A), shows that using a mixture of surfactants does not have a maximum delay in inversion at intermediate stirring speeds. This is due to easier and quicker diffusion of smaller chains of surfactant (Igepal CO-520) which makes the system a non diffusion-controlled process.

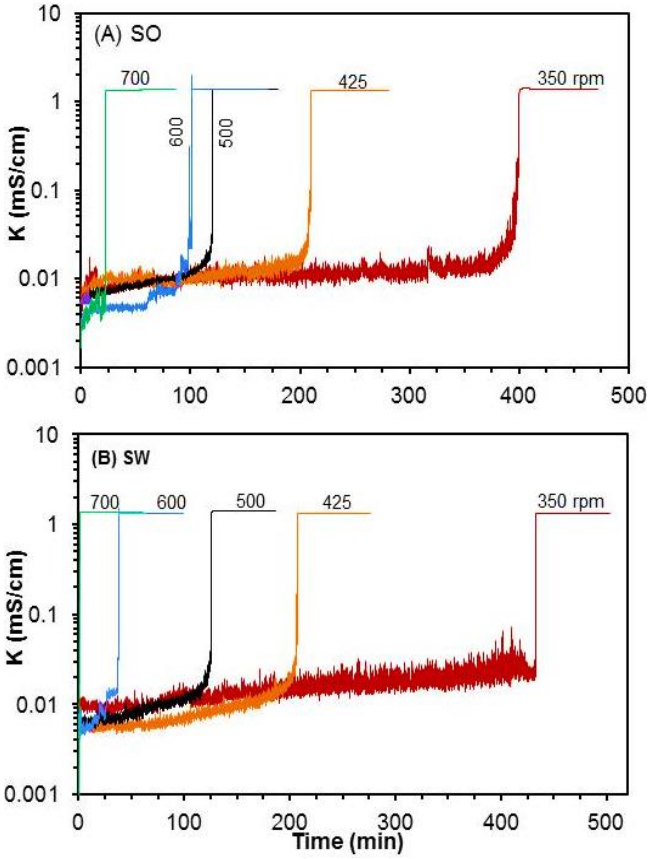


Figure 3.12. Time evolution versus conductivity at different stirring speeds for CPI (SO), (HLB = 13, [S] = 5.0 wt.%, f_w) = 0.25, T = 25ºC, [KCl] = 0.5 wt.%).

Figure 3.13(B) indicates the effect of stirring speed on average drop size $D_{3,2}$ using mixed surfactants (HLB13). As the stirring speed increases, $D_{3,2}$ decreases for all runs. Both direct and catastrophic phase inversion show the same behaviour, but the drop size for the direct method is bigger than that for the CPI method. The reason for this, as stated above, is that at high stirring speeds the drop break-up rate is greater than the drop coalescence rate. Thus, the surface area increases and the interfacial tension decreases, producing small drops (Salager et al., 2002). This figure also reveals that the drop size of the final

emulsion for both methods is highly affected by the surfactant location. However, at low stirring speeds, the difference in drop size between the two methods is higher than that at higher stirring speeds. This means that the effect of the stirring speed is more important at lower values than at higher values.

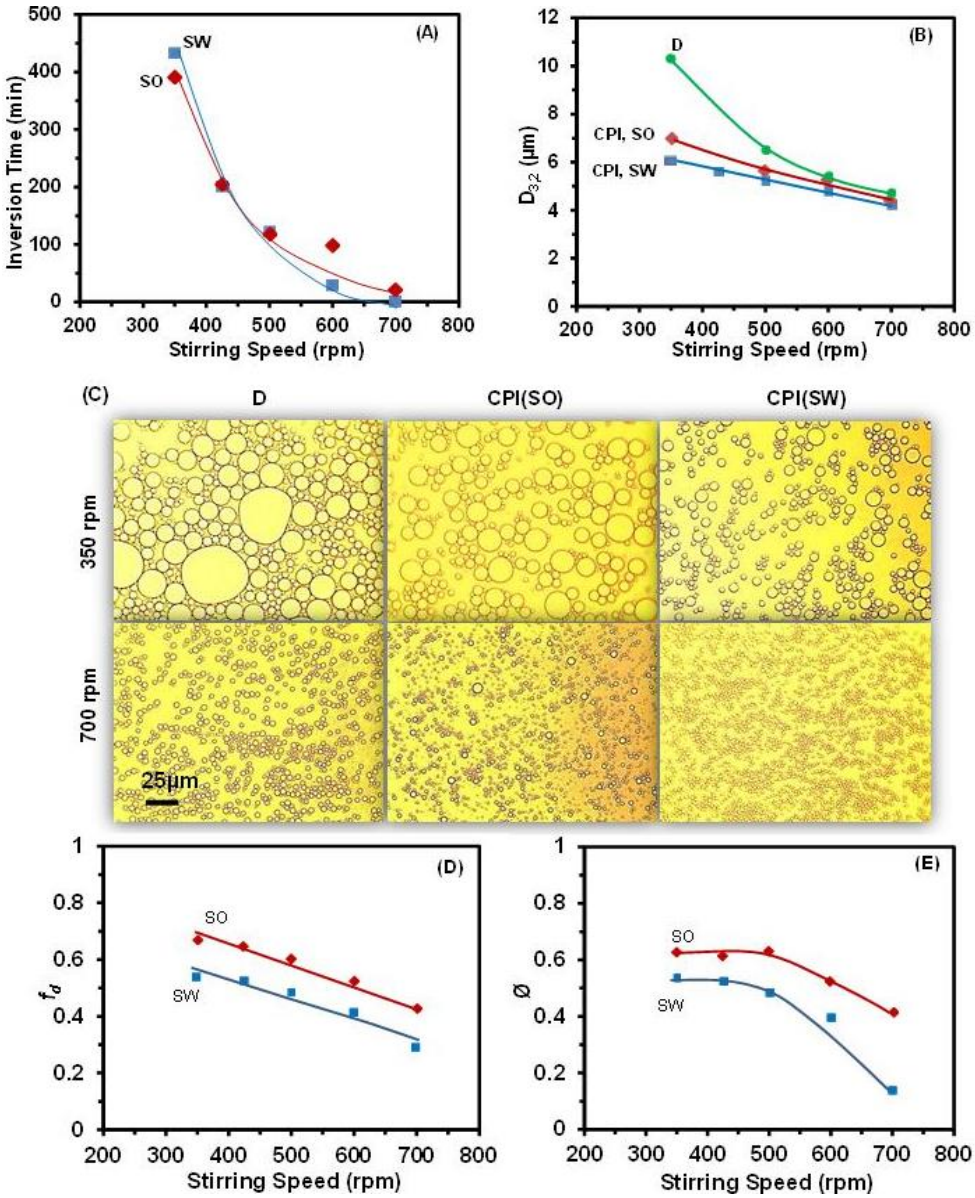


Figure 3.13. A) Inversion time, and B) $D_{3,2}$ versus stirring speed for CPI, C) Corresponding micrographs, (D and E) Effective dispersed phase fraction and internal phase fraction versus stirring speed at inversion time, (HLB = 13, f_w) = 0.25, T = 25ºC, [S] = 5.0 wt.%, [KCl] = 0.5 wt.%).

Micrographs of the final emulsions produced for this set are shown in Figure 3.13(C), which supports the above findings. This figure shows the existence of some droplets; their shape is not a sphere when a low stirring speed is used. This also shows that at a low stirring speed the drop size of the emulsions produced is bigger than the drop size at a high stirring speed; the CPI method produces

smaller drops than the D method. The results are similar to the results obtained using Mastersizer laser diffraction.

Figure 3.13(D and E) shows the effect of stirring speed on f_d and Ø at inversion time. The results show that f_d and Ø are decreasing as stirring speed increasing for both SO and SW. Figure 3.13(E) shows the effect of stirring speed on Ø at inversion time. As it is clear, there is no effect of stirring speed on Ø at lower values of stirring speed below 400 rpm; however, beyond this point Ø starts to decline. For the surfactant dissolved in the oil phase both f_d and Ø are higher than if the surfactant dissolved in the water phase.

3.1.2.4. Temperature

In order to study the effect of the temperature on the CPI, three sets of experiments were conducted using different surfactant HLBs at the temperature range between 25°C and 65°C.

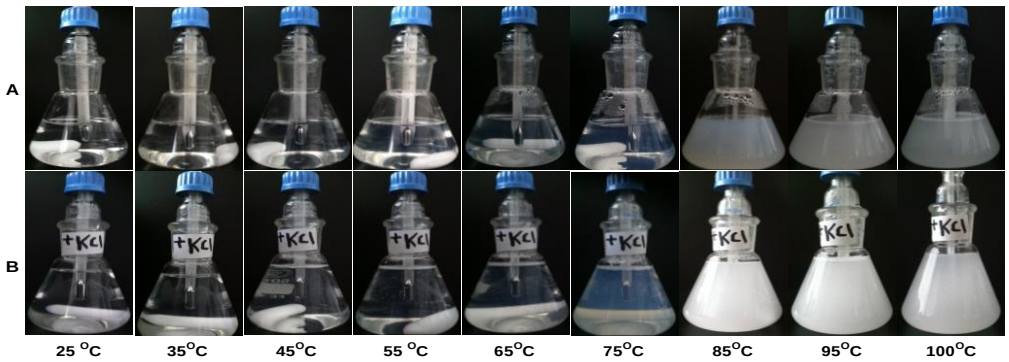


Figure 3.14. Effect of the temperature on the solubility of the surfactant Igepal CO-720; A) without KCl, B) with 0.25 g KCl. (2.5g of the surfactant dissolved in 50 ml of distilled water.)

Prior to the investigation of the temperature effects on the CPI emulsification, we carried out two simple experiments to study the effect of the temperature on the solubilisation capacity of non-ionic surfactants and the effect of the additives (KCl in this case) on their cloud point. Igepal CO-720 was selected for this purpose. The solubilisation capacity of the non-ionic surfactant (CO-720) aqueous solution was determined visually by increasing the temperature at which the cloud point was observed visually. This is in agreement with the previous findings that non-ionic surfactants become more oil soluble with increasing temperature (Chai and Mu, 2002). The effect of KCl on the cloud point was also investigated. It was found that the cloud point decreases from 50°C to 45°C by adding 0.25 g KCl to the surfactant solution, see Figure 3.14.

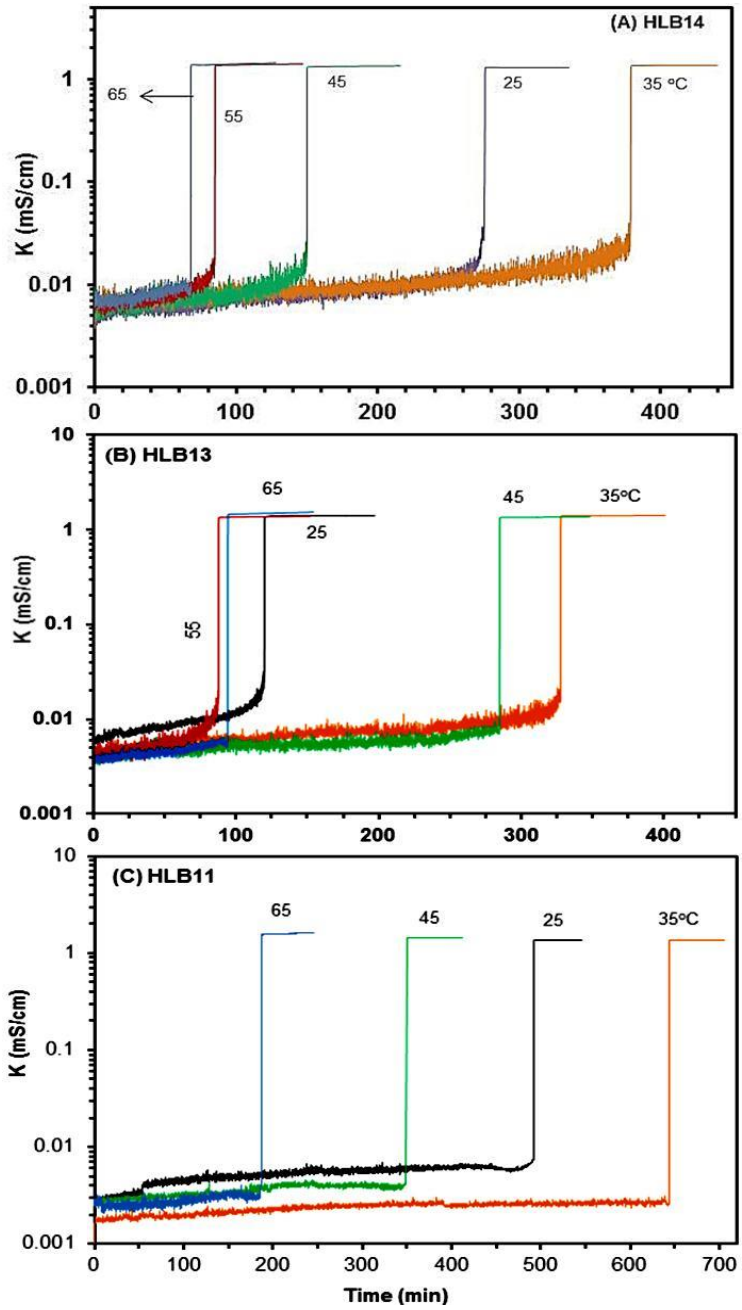


Figure 3.15. Time evolution versus conductivity for CPI (SO) at different temperatures for surfactant HLB of A) 14.0, B) 13.0, and C) 11.0. (Stirring speed = 500rpm, f_w = 0.25, [S] = 5.0 wt.%, [KCl] = 0.5 wt.%).

Figure 3.15(A), (B) and (C) show the time evolution of conductivity during CPI at different temperatures for HLB of 14, 13, and 11, respectively. These figures show that the extent of inclusion decreases with increasing the temperature of the system (except for T=25°C). As temperature increases surfactant becomes more hydrophobic, i.e. the HLB of the surfactant decreases. As explained before, decreasing the HLB of the surfactant will lead to a decrease in the extent of inclusion. Looking at this figure carefully one can see that the noises of the

conductivity measurements diminishes as HLB of the surfactant decreases. This is because the stability of the abnormal system at lower HLB (i.e., closer to the optimum LB) toward inversion is improved in comparison to the stability at higher HLB.

Figure 3.16(A) represents the variation of the inversion time for the O/W emulsions prepared via CPI at different temperatures. For each of the three sets conducted, phase inversion time has a maximum value at T=35°C. It is not clear why the inversion time at 25°C is lower than that at 35°C. But above this temperature, the inversion time starts to decrease to its lowest value at 65°C. The reason for decreasing inversion time above 35°C could be related to the cloud point of the surfactant(s). By increasing the temperature of the system the surfactant becomes more hydrophobic, i.e. its solubility increases in the oil phase. This figure also shows that the difference in inversion times for different HLBs is more important at lower temperatures. Also the trend at low temperatures is in agreement with Figure 3.9(A), which indicates a minimum inversion time at HLB of around 13 in comparison to higher HLB of 14 or lower HLB of 11, and longest inversion was observed at HLB=11. Figure 3.16(A) also shows the longest inversion times belong to HLB=11. The influence of the temperature on $D_{3,2}$ is demonstrated in Figure 3.16(B). As expected, increasing the temperature of the system will lead to a decrease in $D_{3,2}$ of the final emulsion. The major difference occurs at the lowest temperature. Interestingly, all sets produce the same drop size at T=65°C. Another observation in this figure is that the drop size decreases as the surfactant HLB decreases; as stated earlier, this is due to the reduction in the size of the internal oil droplets. Increasing the temperature of the system will increase the hydrophobicity of the surfactants, i.e. HLB of the surfactants decreases. In other words, the affinity of the surfactant mixture toward the oil phase increases. This is associated with a decrease in the interfacial tension, which favours more break-ups (Sajjadi et al., 2003a). The results illustrated in this figure are in agreement with the results illustrated in Figure 3.9(B), the effect of HLB on $D_{3,2}$. For both methods, D and CPI, $D_{3,2}$ is increases with HLB, however, CPI produces finer drops. Micrographs of the emulsions produced at different temperatures are shown in Figure 3.16(C). The images show that the HLB of the surfactant has no significant effect on the drop size of the final emulsion at higher temperatures.

Figure 3.16(D) and (E) show the effective dispersed phase fraction and the internal phase fraction, respectively, at inversion time. As can be clearly seen, both f_d and Ø decrease as the temperature of the system increases (i.e., HLB decreases). The results show that at lower temperaturesthe f_d is affected significantly by HLB, while higher temperatures have almost no effect. The same trend, but with less order of magnitude, can be seen on the effect of temperature on Ø.

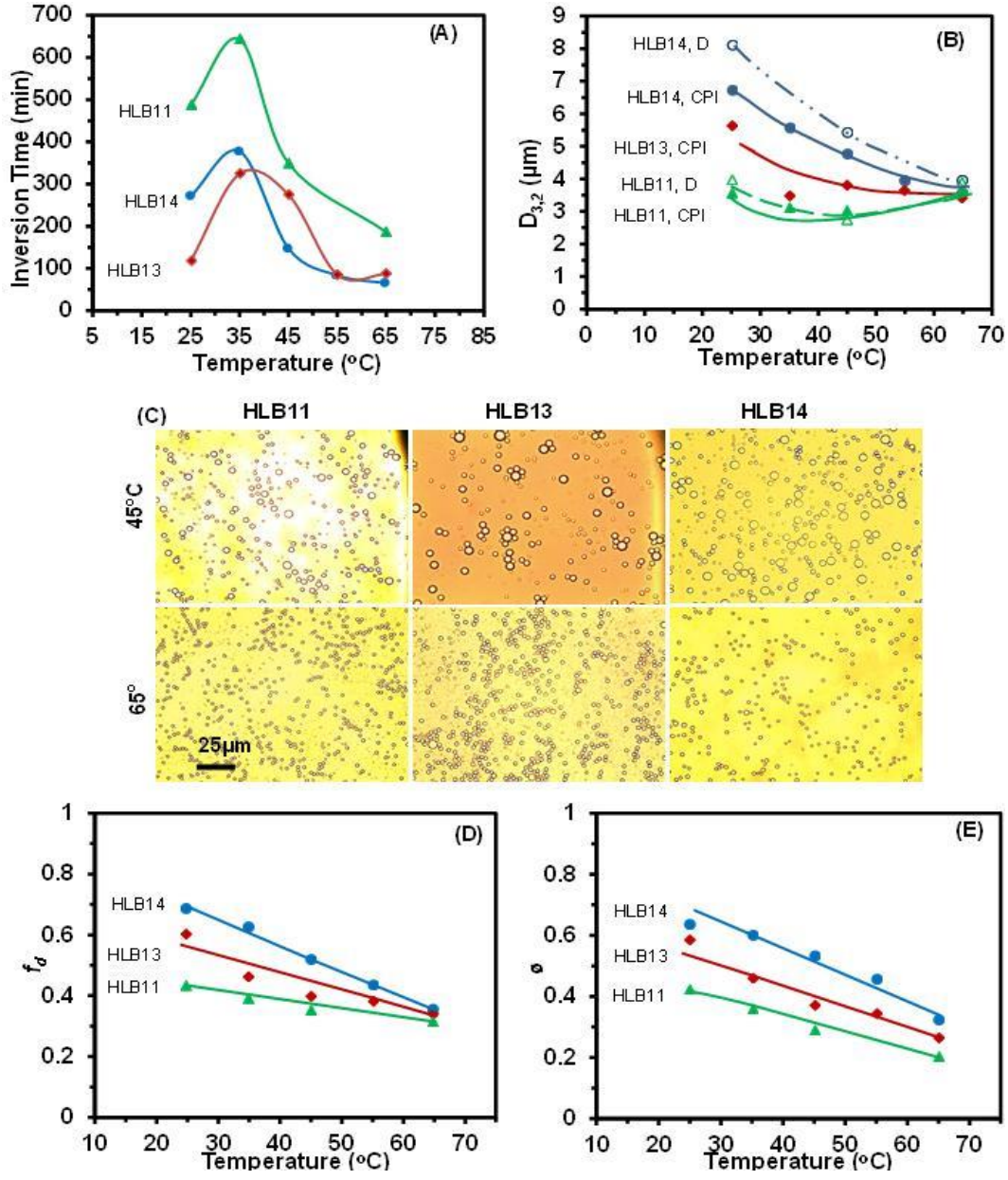


Figure 3.16. A) Inversion time, and B) $D_{3,2}$versus temperature, C) Corresponding micrographs, (D and E) Effective dispersed phase fraction and internal phase fraction at inversion time versus temperature, for CPI (SO) at different HLBs (Stirring speed = 500rpm, f_w) = 0.25, [S] = 5.0 wt.%, [KCl] = 0.5 wt.%).

3.1.2.5. General Conclusion

Inclusion is the core mechanism of inversion of emulsions via CPI emulsification. Process and formulation variables affect the rate and extent of the inclusion, which in turn affect the inversion time and drop size.

CPI emulsification method is capable of producing finer emulsions in comparison to direct emulsification method mainly when the inclusion is extensively happens leading to formation of smaller inner droplets, which in turn form the dispersed phase in the inverted emulsion. (see Chapter 4 for detailed Conclusions.)

3.2. Multiple Emulsions

In this section, the results of study of effects of different parameters on emulsion stability (encapsulation efficiency) during preparation of multiple emulsions are presented.

First, the mechanism of release of inner phase to the external phase is discussed and based on that a simple model is developed in order to predict the encapsulation efficiency of multiple emulsions using the conductivity measurements. Then, the effect of different parameters, such as stirring speed, surfactant concentration and phase fraction, are discussed.

3.2.1. Release Mechanisms

As previously mentioned in the literature review, there are mainly two mechanisms via which the inner phase (here an ionic solution) can be released during secondary emulsification. They are 1) diffusion via reverse micelles through the membrane, and 2) the coalescence of internal droplets with the external phase or membrane rupture, which leads to droplet exit (Florence et al., 1985). These are illustrated in Figure 3.17(A).

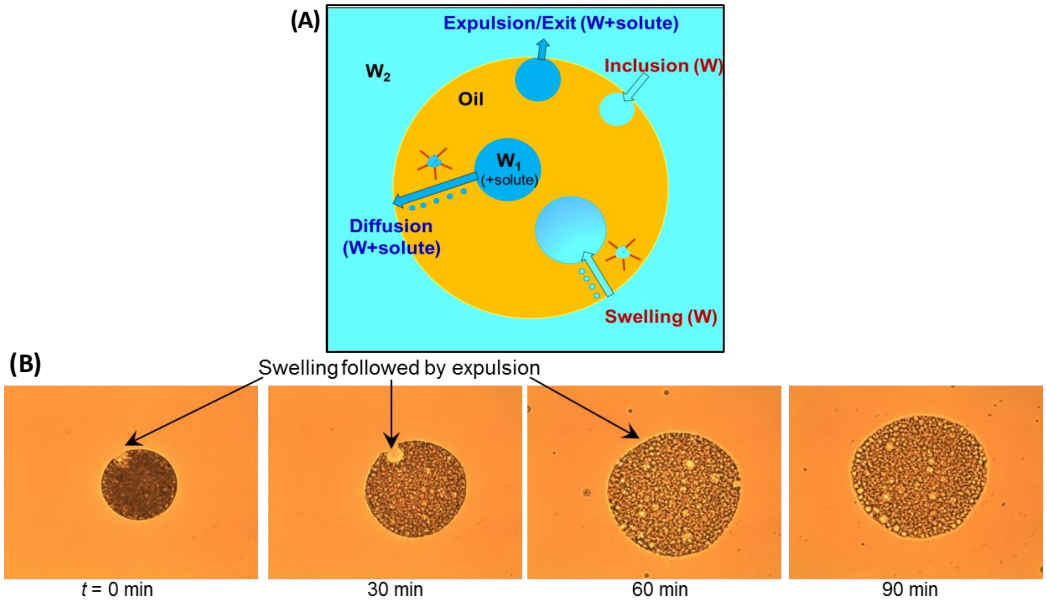


Figure 3.17. (A) The schematic illustration of destabilisation mechanisms. (B) Change in the morphology of a single multiple drop with time at base conditions (Data in B from Paramalingam, 2012).

1) Diffusion via micelles: The diffusion of ions through inverse micelles occurs via the following three stages: i) Reverse micelles containing ions are formed by the internal water droplets. This requires ions to cross water droplets-oil interface,

diffuse through oil phase boundary, cross the micelle interface and then diffuse into the core of micelles; ii) Micelles have to diffuse through the oil phase towards the oil-external drop interface; iii) Micelles are unloaded with ions at the interface. Assuming that the formation of micelles, uploading and downloading is within milliseconds and very fast (Aserin, 2007), the diffusion time of reverse micelles in the oil phase, t_{diff}, is the controlling stage that can be represented by:

$$t_{diff} = \frac{L^2}{4D} \quad (3.3)$$

Where *L* is the average distance the micelles have to travel inside the oil drop to reach to the interface, and *D* is the diffusion coefficient given by Stoke-Einstein equation as:

$$D = \frac{k_B T}{6\pi\mu r} \quad (3.4)$$

Where μ is the viscosity of the oil phase, *T* is the temperature, *r* the radius of reverse micelles and k_B is Boltzmann constant (1.38×10^{-23} J/K). The viscosity of the oil was measured to be about 0.7 Pa.s. The radius of the Span 80 micelles is estimated to be (at least) around 25 nm (Qiong, 2012). Considering the room temperature, 20°C, and using equation 3.4 the diffusion coefficient was calculated to be:

$$D = 1.23 \times 10^{-15} \mathrm{m}^2/\mathrm{s}.$$

To estimate *L*, we consider the average diameter of oil drops to be $60\mu\mathrm{m}$ (according to the results of this study for the base conditions) and the internal water phase fraction as 0.3 (the base value for $\emptyset_{1,0}$). Assuming one single water droplet, the average distance of diffusion is estimated as $L = 10\ \mu\mathrm{m}$. (One should note that droplets closer to the oil-external water interface has a smaller distance for diffustion. However, those droplets closer to the center of the oil drop has longer diffusion distance.)

Therefore, using equation 3.3, t_{diff} is calculated to be about 30 min. (In fact, for $L = 3\ \mu\mathrm{m}$, we will have $t_{diff} = 3\ \mathrm{min}$.)

Comparing the calculated value of t_{diff} with the emulsification time of 2 min used in this study, one can realise that the two mechanisms occur over a time scale significantly different so that can be decoupled. This is also consistent with literature that show release by diffusion occurs at long time scales (Aserin, 2007).

2) Membrane rupture, coalescence or exit: Exit is a mechanism by which a droplet leaves a drop. Exit appears to be more important during emulsification when drops are exposed to high shear and are deformed so that the internal droplets expelled from the drop.

The extent of release through exit is related to the fraction of the water phase that has entered the external continuous phase. The initial fraction of the dispersed phase is set by the emulsion composition. However, there are two phenomena that contribute to increasing effective dispersed phase.

i) Osmosis driven swelling: Multiple emulsions tend to undergo swelling due to osmotic pressure induced by concentration difference of substances in the most inner and outer phases. For W/O/W emulsions containing a solute in the inner phase, water maymigrate from the continuous phase to the inner water droplets. Usually a water-soluble gradient such as glucose is added to the external water phase to balance the osmotic pressure induced by the presence of salt in the internal water phase. However, this may affect the finalproperties of the resulting emulsions. We did not use any ingredient in the external phase to balance the osmotic pressure. We monitored drop size evolution for a number of multiple emulsions after preparation. A typical growth of multiple drops due to osmotic pressure is shown in Figure 3.17(A). It is clear from these micrographs that the growth of globules and internal droplet coalescence occur over time. The globule size increased from 64.8 to 68.4 within 10 min, which is actually less than 4% increase in volume within 2 min. Therefore this instability mechanism can be safely ignored in the course of preparation of multiple emulsions.

ii) Inclusion: Multiple emulsions are reconciled mixture of two opposite emulsions in one emulsion as they contain both water-soluble and oil-soluble surfactants. The oil-soluble surfactant tends to stabilise W/O emulsions and water-soluble surfactant tends to stabilise O/W emulsions. When the primary W/O emulsion is added to the secondary water phase to form W/O/W multiple emulsions, it is likely that the oil phase includes some water from the external water phase. The mechanism of inclusion has been attributed to drop deformation in the presence of surfactants (Aserin, 2007).

The droplet inclusion combined with possible coalescence with other internal droplets inside the oil drop and droplet exit make tracking even more

complicated. This suggests that the change in the effective phase fraction may not be directly related to the release.

3.2.2. Inclusion

In order to evaluate inclusion in the course of preparation of multiple emulsions, we conducted a number of emulsification using single O/W emulsions with the same overall recipe as that for the multiple emulsions but with different Span 80 concentrations in the oil phase (i.e., [S_1]). The water continuous phase contained a salt concentration of 0.013M (calculated assuming all the internal aqueous phase has been released to the external aqueous phase). The inclusion, change in effective phase fraction, was calculated from conductivity readings (*K*) using Bruggeman's law for single emulsions:

$$K = K_w \emptyset_w^{3/2} \qquad (3.5)$$

where K_w is the water phase conductivity (measured separately for each experiment) and $\emptyset_w$ is the water phase fraction.

The results can be seen in Figure 3.18(A) and the micrographs in Figure 3.18(B), which clearly shows that oil drops contained some water droplets at the end of emulsification.It should be mentioned here that the effective phase fractions for multiple emulsions were estimated using equation 3-15, i.e., the derived 'modified Bruggeman's law' for multiple emulsions.

The inclusion was significant for [S_1] > 0.5%. However, the emulsions used for the study of inclusion were single O/W emulsions. To see the effect of the internal phase fraction of the primary emulsion on inclusion, a number of multiple emulsions were prepared at different primary phase fractions, $\emptyset_{1,0}$, with no salt in the inner and outer phases, as seen in Figure 3.18(E). This allowed us to relate the change in the emulsion conductivity during emulsification to the change in the internal phase fraction (i.e., inclusion). The results clearly show that inclusion reduced with increasing $\emptyset_{1,0}$. For $\emptyset_{1,0}$=0 (i.e., single emulsions) inclusion was significant, as previously stated. For the run with $\emptyset_{1,0}$=0.1, however, inclusion occurred in the first approximately 20 seconds, but later on the droplet exit overcame as effective phase fraction continued to decrease. For $\emptyset_{1,0}$>0.1, either inclusion did not occur or if it did, it was overcame by the rate of exit of droplets. This analysis can be supported by the drop size results shown in Figure 3.18(C) and the micrographs seen in Figure 3.18(D). Therefore, the results suggests that

the importance of inclusion may be less for double emulsions than single emulsions due to the presence of internal drops. It also suggests that in the absence of slat in the inner and outer phase, droplets tend to exit the oil drops.

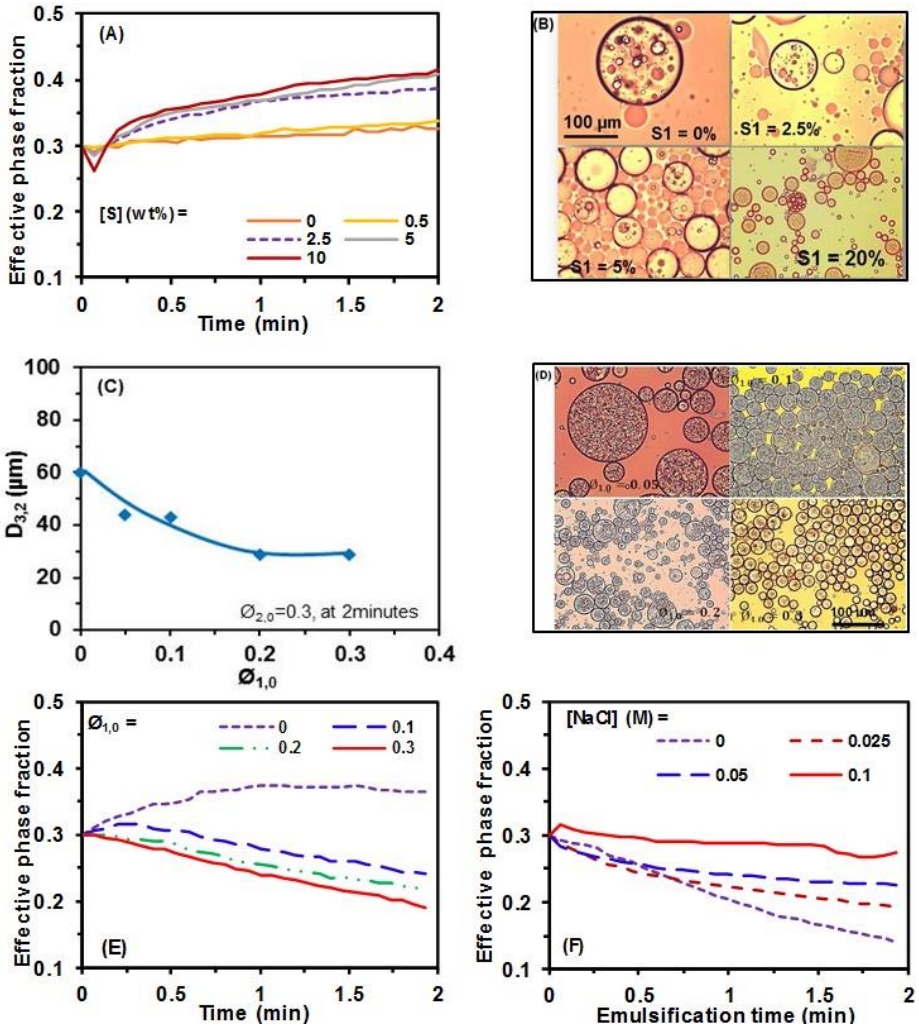


Figure 3.18. A) Effective phase fraction, B) Micrographs (at t=2min), of single emulsions versus emulsification time, for different $[S_1]$, ($Ø_{2,0}$ = 0.30, N_2 = 3400 rpm, $[S_2]$ = 2%, [NaCl] = 0.013 M in water); C) $D_{3,2}$, D) Micrographs (at t=2min); E), Effective phase fraction of multiple emulsions versus emulsification time at different $Ø_{1,0}$, ($Ø_{2,0}$ = 0.30, N_1 = 9400 rpm, N_2 = 3400 rpm, $[S_1]$ = 5%, Tween 80 $[S_2]$ = 2%, [NaCl] = zero M; F) Effective phase fraction of multiple emulsions versus emulsification time at different [NaCl], ($Ø_{1,0}$ = 0.30, $Ø_{2,0}$ = 0.30, N_1 = 9400 rpm, N_2 = 3400 rpm, $[S_1]$ = 5%, ($[S_2]$ = 2%).

The next step was to study the effect of slat concentration in the inner water droplets on their stability while the outer phase did not contain any salt.

Figure 3.18(F) shows the effect of salt concentration in the inner phase on the effective phase fraction during emulsification, for more details see appendix(B) Figure 7.2. The presence of the salt in the inner phase led to the stability of internal droplets; droplets with high concentration of salt showed little tendency

for exit. These results are broadly consistent with other studies, which have shown that release of internal phase decrease as the concentration of salt increase in the internal phase (Magdassi and Garti, 1984; Hattrem et al., 2014). The existence and the concentration of electrolytes have a significant effect on the stability of W/O and W/O/W emulsions by balancing the Laplace pressure differences between the inner water droplets, and by playing a critical role in balancing osmotic pressure effects between the two water phases (Rosano et al., 1998). In the absence of salt, around 60% of the inner phase was released to the outer phase within 2 min, which can only be accounted for by droplet exit. The most stable multiple emulsion was obtained at [NaCl] = 0.10 M.

By the same token, it can be concluded that drops included from the continuous phase, which have no salt, have little tendency to remain in the oil drops, after any possible inclusion event, and will leave the oil phase quickly.

Overall, for $[S_1]$<10% and when the salt concentration in the outer phase was nil, the typical inclusion was reduced to lower than 4% which could be safely ignored. For this reason, we limited the $[S_1]$ below 10% in this study. We can also conclude that results for the most stable double emulsions made, which did not allow any salt release during emulsification, were the most accurate.

Having eliminated alternative mechanisms of release and the conditions that could allow inclusion to occur, we can now define the droplet exit process as the main mechanism for the release of the salt during the course of preparation of multiple emulsions. The effective volume fraction of the dispersed phase is reduced during release and that reduction can be correlated with the change in conductivity. Based on the above analysis we developed a simple model, to predict the encapsulation efficiency of freshly-prepared multiple emulsions.

3.2.3. Model development

The purpose of developing a mathematical model is to predict the release and encapsulation efficiency from the conductivity measurements.

If $\emptyset_{1,0}$ and $\emptyset_{2,0}$ are the initial primary and secondary emulsions phase fractions, respectively, we will have:

$$\emptyset_{1,0} = \frac{W_1}{W_1+O} \quad (3.6)$$

$$\emptyset_{2,0} = \frac{W_1+O}{W_1+W_2+O} \quad (3.7)$$

Where *O* is the amount of oil in the recipe and W_1 and W_2 are the amounts of water in primary and secondary emulsions, respectively. Defining encapsulation efficiency, EE, as the fraction of initial internal water phase which has remained in multiple drops, the multiple emulsion phase fraction ($\emptyset_2$) can be defied as:

$$\emptyset_2 = \frac{EE.\mathrm{W}_1+\mathrm{O}}{\mathrm{W}_1+\mathrm{W}_2+\mathrm{O}} = \frac{\mathrm{W}_1+\mathrm{O}}{\mathrm{W}_1+\mathrm{W}_2+\mathrm{O}} - (1-EE)\frac{\mathrm{W}_1}{\mathrm{W}_1+\mathrm{W}_2+\mathrm{O}} \quad (3.8)$$

Using equations 3.6 and 3.7 we can rewrite equation 3.8 as:

$$\emptyset_2 = \emptyset_{2,0} - (1-EE)\emptyset_{1,0}\emptyset_{2,0} \quad (3.9)$$

which can be rearranged for EE:

$$EE = 1 - \frac{\emptyset_{2,0}-\emptyset_2}{\emptyset_{1,0}\emptyset_{2,0}} \quad (3.10)$$

Equation 3.10 reveals a linear relationship between the encapsulation efficiency, *EE*, and the multiple emulsion phase fraction, $\emptyset_2$.

If all the internal water phase is released to the external aqueous phase (i.e., EE=0) the minimum multiple emulsion phase fraction ($\emptyset_{2\mathrm{min}}$) can be obtained from equation 3.8. This is simply the phase fraction for a single emulsion resulting from total release of the internal water phase:

$$\emptyset_{2,min} = \emptyset_{2,0}(1-\emptyset_{1,0}) \quad (3.11)$$

The salt concentration in the external phase can be given by the following equation:

$$C = \frac{(1-EE)\mathrm{W}_1 C_0}{(1-EE)\mathrm{W}_1+\mathrm{W}_2} \quad (3.12)$$

Where C_0 is the initial salt concentration in primary water phase. Combining equations 3.6, 3.7, and 3.12 results in:

$$\frac{C}{C_0} = \frac{(1-EE)\emptyset_{1,0}\emptyset_{2,0}}{(1-EE)\emptyset_{1,0}\emptyset_{2,0}+1-\emptyset_{2,0}} \quad (3.13)$$

Finally substituting EE by equation 3.8 results in the following simple equation:

$$\frac{C}{C_0} = \frac{\emptyset_{2,0}-\emptyset_2}{1-\emptyset_2} \quad (3.14)$$

In order to find a correlation between the conductivity of the multiple emulsion and the encapsulation efficiency, one may start from the Bruggeman's law for single emulsions; $K = K_w\emptyset_w^{3/2}$ (equation 3.3) where K and K_w are the conductivities of emulsion and water phase, respectively, and $\emptyset_w$ is the water volume fraction. The conductivity of water phase, K_w, may be considered as a linear function of the salt concentration, C, with slope of k (i.e., K_w=kC, see the inset of Figure 3.19). Also, it is clear that $\emptyset_w = 1 - \emptyset_2$, therefore:

$$K = kC(1-\emptyset_2)^{3/2} \quad (3.15)$$

The above equation could be considered as a modified Bruggeman's law for multiple emulsions. Substituting C and $\emptyset_2$ using equations 3.12 and 3.7 will result in:

$$K = kC_0(1-EE)\emptyset_{1,0}\emptyset_{2,0}(1-\emptyset_{2,0}+(1-EE)\emptyset_{1,0}\emptyset_{2,0}))^{1/2} \quad (3.16)$$

In the above equation the term kC_0 indicates the conductivity of the primary emulsion aqueous phase. Calling it K_{w0}, this equation can be rewritten as:

$$K = K_{w0}(1-EE)\emptyset_{1,0}\emptyset_{2,0}(1-\emptyset_{2,0}+(1-EE)\emptyset_{1,0}\emptyset_{2,0}))^{1/2} \quad (3.17)$$

Equation 3.17 gives a relationship between the conductivity of the multiple emulsion, K, and the encapsulation efficiency, EE. In order to validate this correlation, it was reduced to the case with 100% release of internal water phase (i.e., EE=0):

$$K = K_{w0}\emptyset_{1,0}\emptyset_{2,0}(1-\emptyset_{2,0}+\emptyset_{1,0}\emptyset_{2,0})^{1/2} \quad (3.18)$$

The validity of equation 3.18 was tested against experimental results. A series of single O/W emulsions were prepared each representing a multiple emulsion assuming all the internal phase has been released to the external aqueous

phase. These single emulsions were prepared considering a range of values for $\emptyset_{1,0}$(0.3, 0.5, and 0.7) and $\emptyset_{2,0}$ (0.1-0.8 with 0.1 increments). The conductivity of each single emulsion was measured and plotted against the r.h.s. of equation 3.16 as seen in Figure 3.19. This figure demonstrates a very good agreement between experimental results and the Bruggeman's law modified for multiple emulsions.

According to equation 3.17, in case of no release (i.e., EE=1), the conductivity of the multiple emulsion, K, would be zero which represents the conductivity of the external deionised water phase which is very low and can be ignored. Also in the case of very dilute multiple emulsions, $\emptyset_{1,0} = \emptyset_{2,0} = 0$, the conductivity predicted by the model is that of the deionised water.

From the above analysis, it is concluded that equation 3.17 can be used to estimate the encapsulation efficiency, EE, using the conductivity of the multiple emulsion, K, and that of the internal phase (aqueous salt solution), K_{w0}, and the phase fractions of primary ($\emptyset_{1,0}$) and secondary ($\emptyset_{2,0}$) emulsions. Equation 3.17 can be solved iteratively. Alternatively, it can be rearranged for EE to give a cubic equation and the real root may be calculated which is not discussed here.

For example, for the experiment conditions of $\emptyset_{1,0} = 0.3$, $\emptyset_{2,0} = 0.3$, $N_1 = 9400\,rpm$, $N_2 = 3400\,rpm$, $[(S_1] = 5\%$, $[S_2] = 2\%$, and NaCl=0.1 M, the conductivity of the freshly-prepared multiple emulsion was measured as $K = 0.0183\,mS/cm$, and knowing K_{w0}=11.04 mS/cm (see caption of Figure 3.19), the best value of encapsulation efficiency to fit equation 3.17 was found to be 97.8%.

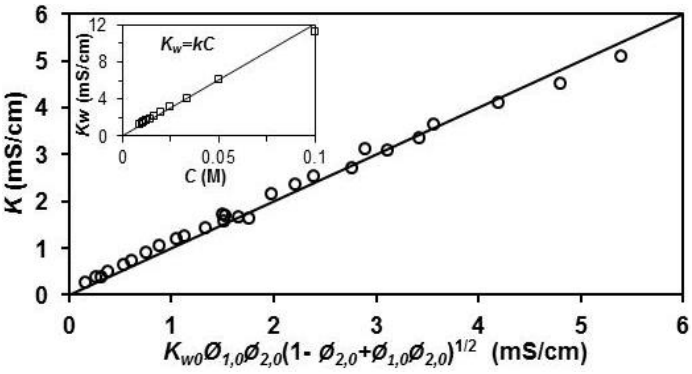


Figure 3.19. Conductivity of multiple emulsions against r.h.s. of equation 3.18. Inset: Conductivity of aqueous NaCl solutions. The slope of the best fitted line is k=110.4 mS/cm.M. For this work: K_{w0}=kC_0 =110.4×0.1=11.04 mS/cm, and different values for $\emptyset_{1,0}$ (0.3, 0.5, and 0.7) and $\emptyset_{2,0}$ (0.1-0.8 with 0.1 increments) were used.

3.2.4. Experimental results and discussion

Multiple (W/O/W) emulsions were prepared using two-step emulsification method. Several parameters were altered to choose the best formulation, which produces the multiple emulsions with higher initial encapsulation efficiency, $EE\%$. The effect of different parameters, including salt concentration; stirring speeds, surfactants concentrations, and phase fractions in both primary and secondary emulsification; and finally the oil viscosity, on encapsulation efficiency and multiple drop size were studied. Tween 80 and Span 80 were selected as the inner and the outer surfactants, respectively, while paraffin oil and deionised water were used as the oil and the water phase, respectively.

Table 3.2 shows the formulation used to prepare multiple emulsions for the base experiment. Subscripts 1 and 2 refer to step 1 (primary emulsification) and step 2 (secondary emulsification), respectively. Ø is the dispersed phase fraction (volume of dispersed phase to the total volume of emulsion), and S represents the surfactant (Span 80 for step 1, Tween 80 for step 2). N represents the stirring speed.

Table 3.2. Formulation used to prepare multiple emulsions.

Step 1: Primary Emulsification				**Step 2: Secondary Emulsification**		
$Ø_1$ (%)	**[S_1] (w/v%)**	**[NaCl] (M)**	**N_1 (rpm)**	**$Ø_2$ (%)**	**[S_2] (w/v%)**	**N_2 (rpm)**
30	10	0- 0.2	9400	33	2	3400
30	10	0.1	3400-9400	33	2	3400
30	10	0.1	8000	33	2	3400-8000
30	2.5-20	0.1	9400	33	2	3400
30	10	0.1	9400	33	0.25-4	3400
30	5	0.1	9400	30-80	2	3400
30-80	5	0.1	9400	30	2	3400

3.2.4.1. Primary stirring speed, N_1

W/O/W multiple emulsions were prepared to study the influence of stirring speed of the primary emulsification N_1 on drop size and encapsulation efficiency of multiple emulsions. The range of the stirring speed of the primary emulsification was between 3400 rpm to 9400 rpm. Due to the increase in the number of small droplets the surface area of the emulsion increases, if the amount of surfactant is not enough to cover all the small droplets, then rising the stirring speed has no effect (Jafari et al., 2008). When there is not enough surfactant present in the interface of the newly formed droplets, the droplets colloid and coalescence

occurs (Florence et al., 1985). Figure 3.20(A) show the encapsulation efficiency of multiple emulsions versus N_1. It is evident from Figure 3.20(A) that minimum encapsulation, in other word maximum release, occurs when $N_1 \approx N_2$; i.e., the mixing condition under which the size of internal droplets keep their original size during secondary emulsification, which increases the chance of drop-drop coalescence that leads to release. The release could occur as a result of diffusion or droplet breaking. Both processes can lead to destabilisation of the emulsion. The optimum operation condition, at which minimum release occurs, is then when $N_1 \gg N_2$. This specifies the conditions where $d_{w/o} \approx d_{w/o/w} << D_{3,2}$.

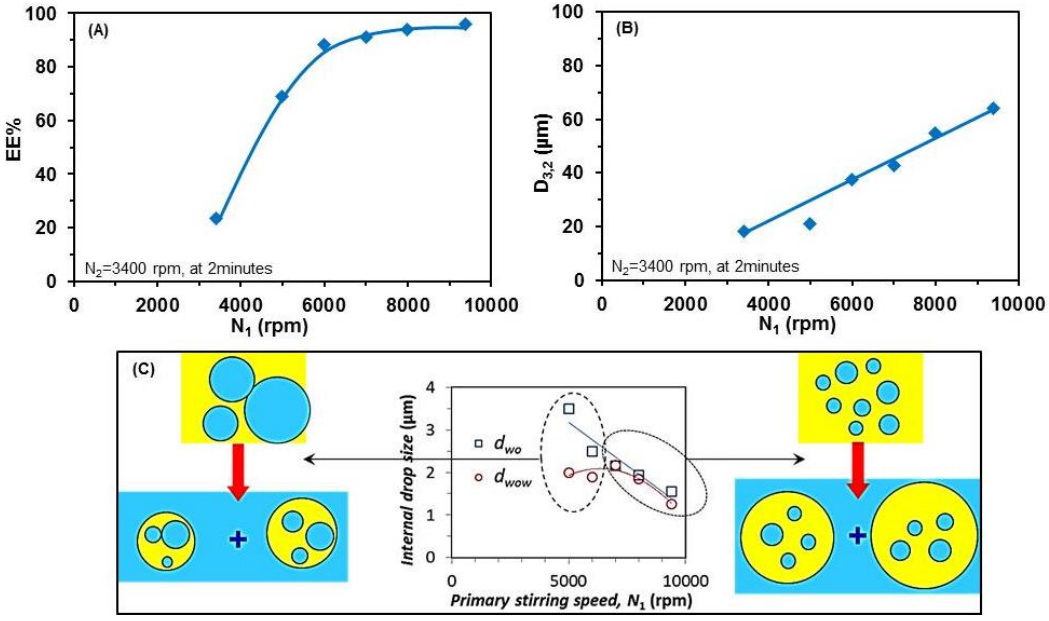


Figure 3.20. (A) Encapsulation efficiency, of multiple emulsion versus N_1, ($Ø_{1,0}$ = 0.30, $Ø_{2,0}$= 0.33, N_2= 3400 rpm, [S_1] = 10%, [S_2] = 2%, [NaCl] = 0.1 M), B) $D_{3,2}$ of multiple emulsion versus N_1, C) The internal drop size $d_{w/o}$ and $d_{w/o/w}$ against N_1, (Data in (C) from Paramalingam, 2012).

Figure 3.20(C) shows variations in the size of internal water droplets, both in the primary W/O and secondary W/O/W emulsions, versus stirring speed of primary emulsifications. It is evident from this figure that the size of internal water droplets decreases with increasing N_1.

Figure 3.20(C) also shows that the small water droplets formed at high N_1did not further broken up during secondary emulsification so that $d_{w/o} \approx d_{w/o/w}$. However, the difference between drop sizes gradually becomes visible with decreasing N_1, indicating that further rupturing of the oil droplets occurs during the secondary emulsification so that $d_{w/o} > d_{w/o/w}$. This may happen at low N_1 where large water droplets are formed. This is the region where large water drops are formed during

primary emulsification. Therefore, the optimum stirring speeds, N_1, are those that do not cause any drop rupture in the course of secondary emulsification so that $d_{w/o} \approx d_{w/ow}$. This latter condition can only be met at high values of N_1.

Micrographs shown in Figure 3.21(A) show the primary W/O emulsions droplets. It is clear that the drop size of the primary emulsions decrease as stirring speed increases as expected. The drop size is reduced by increasing the amount of energy supplied during emulsification (on the assumption that there is sufficient emulsifier to cover new interface and re-coalescence is prevented as much as possible) (Jafari et al., 2008). Also emulsions with smaller drop size have a higher viscosity at a given phase fraction (Jiao and Burgess, 2008). As a result, the size of multiple drops increased with the stirring speed of the primary emulsification. It is also worth mentioning that at very high primary stirring speeds, N_1 = 8000 rpm and N_1 = 9400 rpm, multiple emulsion drops lose their spherical shape and become worm-like in shape, see Figure 3.21(B). This could be due to the high viscosity of the primary W/O emulsion at very high stirring speeds and surfactant concentration (note that for this set [S_1] = 10%), which leads to formation of elongated multiple drops under the shear in the secondary emulsification stage.

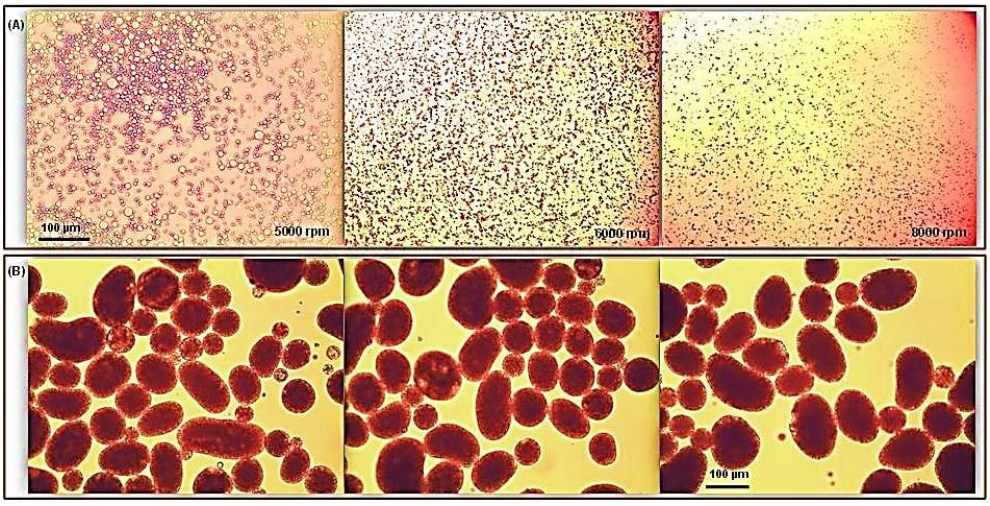


Figure 3.21. Micrographs of (A) Primary, W/O, emulsions at different primary stirring speeds, N_1, (B) Multiple, W/O/W, emulsions at N_1 = 8000 rpm, ($\emptyset_{1,0}$ = 0.30, $\emptyset_{2,0}$= 0.30, N_2= 3400 rpm, [S_1] = 10%, [S_2] = 2%, [NaCl] = 0.1 M).

3.2.4.2. Secondary stirring speed, N_2

From the conductivity data, the encapsulation efficiency after secondary emulsification calculated, which are shown in Figure 3.22(A). In this figure, it can be clearly seen that encapsulation or release is severely affected by the stirring

speed in the secondary emulsification stage, N_2. The minimum encapsulation (i.e., maximum release) occurs when $N_1 \approx N_2$. This is the mixing conditions under which the size of internal droplets becomes similar to that of multiple drops, see Figure 3.20(C). This increases the chance of drop-drop coalescence that leads to release. This also leads to minimum multiple drop size as seen in Figure 3.22(B). The optimum operation condition, at which maximum encapsulation (i.e., minimum release) occurs, is then when $N_2 \ll N_1$. This specifies the conditions where $d_{w/o} \approx d_{w/o/w} << D_{3,2}$. If the internal droplets do not further rupture, then one would expect to see little release of active material during the secondary emulsification. The optimum stirring speeds are therefore associated with the minimum release during secondary emulsification.

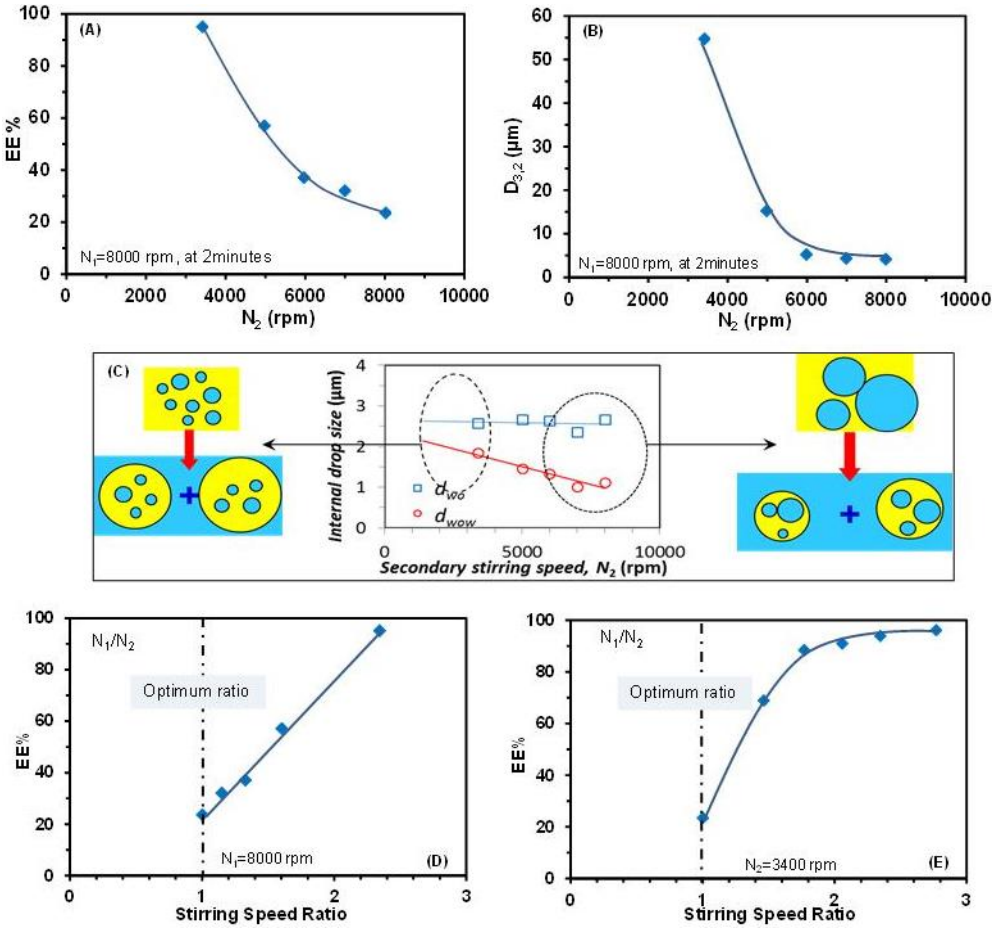


Figure 3.22. (A) Encapsulation efficiency and (B) $D_{3,2}$ of multiple emulsion versus N_2, C) The internal drop size $d_{w/o}$ and $d_{w/o/w}$ against N_2 , ($\emptyset_{1,0}$ = 0.30, $\emptyset_{2,0}$= 0.33, N_1= 8000 rpm, [S_1] = 10%, [S_2] = 2%, [NaCl] = 0.1 M), (D) and (E) Encapsulation efficiency, of multiple emulsion versus stirring speed ratio for N_1 =8000 rpm and N_2 =3400 rpm respectively. (Data in (C) from Paramalingam, 2012).

The size of multiple drops, $D_{3,2}$, produced during secondary emulsification depends on the rates of break up and coalescence of droplets. Increasing the

stirring speed enhances the energy dissipation rate and causes drops to break; hence drops are reduced in size as seen in Figure 3.22(B).

Figure 3.22(C) shows variations in the size of the internal water droplets versus agitation speed of the secondary emulsification. Also, the size of the internal droplets in the multiple droplets decreases with increasing N_2 for the same reason mentioned for $D_{3,2}$. Figure 3.22(D) and (E) demonstrates the effect of stirring speed ratio (N_1/N_2on encapsulation efficiency, which again shows the best (optimum) stirring speed policy is achieved when $N_2 \ll N_1$.

3.2.4.3. Surfactant concentration

The variations in Span 80 concentration, [S_1], at constant Tween 80 concentration ([S_2] = 2.0 wt%) was investigated. It was found that as the concentration of Span 80 increases the drop size of multiple emulsions reduced; see Figure 3.23(A). The change in drop size was not significant when [S_1]>10.0 wt%. Encapsulation efficiency increased with increasing [S_1] as shown in Figure 3.23(B). The CMC of Span 80 is less than 1.0 wt%, and one may assume that the drop size should not change above this value. However, the (external) oil-water interface, which separates the two phases, is affected by the surfactants present in both phases; the outer water phase and the middle oil phase. As a result, the size of multiple drops can be affected by both hydrophobic and hydrophilic surfactants' type and concentration. Note that even though the surfactants were placed in different phases for primary and secondary emulsifications, their possible mixing in the course of emulsification cannot be ignored.

Interfaces with mixed surfactants can show a minimum interfacial tension at an optimum HLB (Garti and Bisperink, 1998; Kanouni et al., 2002). During emulsification, the oil-soluble surfactant dissolved in the oil phase and the water-soluble surfactant dissolved in the water phase quickly diffuse into the oil-water external interface, causing a reduction in the interfacial tension. If the surfactant composition at the interface is close to that of optimum HLB, low interfacial tension promotes drop rupture and formation of small drops.

To make certain that these results are accurate, a series of single O/W emulsions were prepared, and the results were compared with multiple emulsions; see Figure 3.23(A). Single O/W emulsions were prepared under the same conditions of this set of experiments and assuming that all internal water droplets were

released. It was found that $D_{3,2}$ for single emulsions followed the same trend as $D_{3,2}$ for multiple W/O/W emulsions.

Figure 3.23(C) and (D) show the variations in the drop size of multiple W/O/W emulsions and encapsulation efficiency, respectively, against the concentration of Tween 80 in the water phase. The size of multiple drops decreases with increasing the surfactant concentration in water, [S_2]. This was expected as a surfactant can enhance drop rupture by reducing interfacial tension and hinder drop coalescence by sitting at the surface of drops and protecting them against coalescence. Both effects can lead to a reduction in the drop size. However, a minimum drop size is observed when [S_2] = 2.0 wt%. Several reasons can explain the increase in drop size after it reached its minimum value at [S_2] = 2.0 wt%. Note that the critical micellar concentration (CMC) of Tween 80 in water is 7.35 g/l (i.e., 0.735 wt%). Above this level, the multiple drops become fully covered by a surfactant, and their tendency toward coalescence will be minimum too. Increasing [S_2] to 1.0 wt% will only increase the viscosity of continuous phase, without affecting the interfacial tension. This can suppress the turbulency in the system and decrease the drop size, an effect which is probably masked by the concomitant reduction in the interfacial tension if [S] < $[S]_{CMC}$. However, at higher surfactant concentration the viscosity effect can be dominant. It has also been mentioned in the literature that micelles formed in the external water phase can solubilise some of the oil-soluble surfactants (Csóka and Erõs, 1997), and move it to the continuous phase. As a result, the concentration of lipophilic surfactant decreases at the interface of the primary W/O emulsion, which critically affects the stability of multiple droplets by rupture of oil layer and release of the internal drops (Csóka and Erõs, 1997). The increase in drop size can also be explained by the HLB of the multiple emulsions. The droplet sizes are smaller when the HLB value lies between 7 and 9 (i.e., near the optimum HLB) and the HLB values below 7 or above 9 produce larger drops (for example see Figure 3.23(E)). The encapsulation of NaCl during secondary emulsification also decreases with increasing [S_2], as shown in Figure 3.23(D). This is because the increase in Tween 80 concentration enhances rupturing of multiple drops because of the reduced interfacial tension. As a result the encapsulation is reduced (i.e., release is enhanced). It appears that maximum encapsulation/minimum release during secondary emulsification occurs when [S_2] ≤ 1.0 wt%. To validate these results, a series of single O/W emulsions were

prepared, and the results were compared with multiple emulsions see Figure 3.23(C). Single O/W emulsions were prepared under the same conditions of this set of experiments and assuming that all internal water droplets were released. It was found that $D_{3,2}$ for single emulsions followed the same trend as $D_{3,2}$ for multiple W/O/W emulsions.

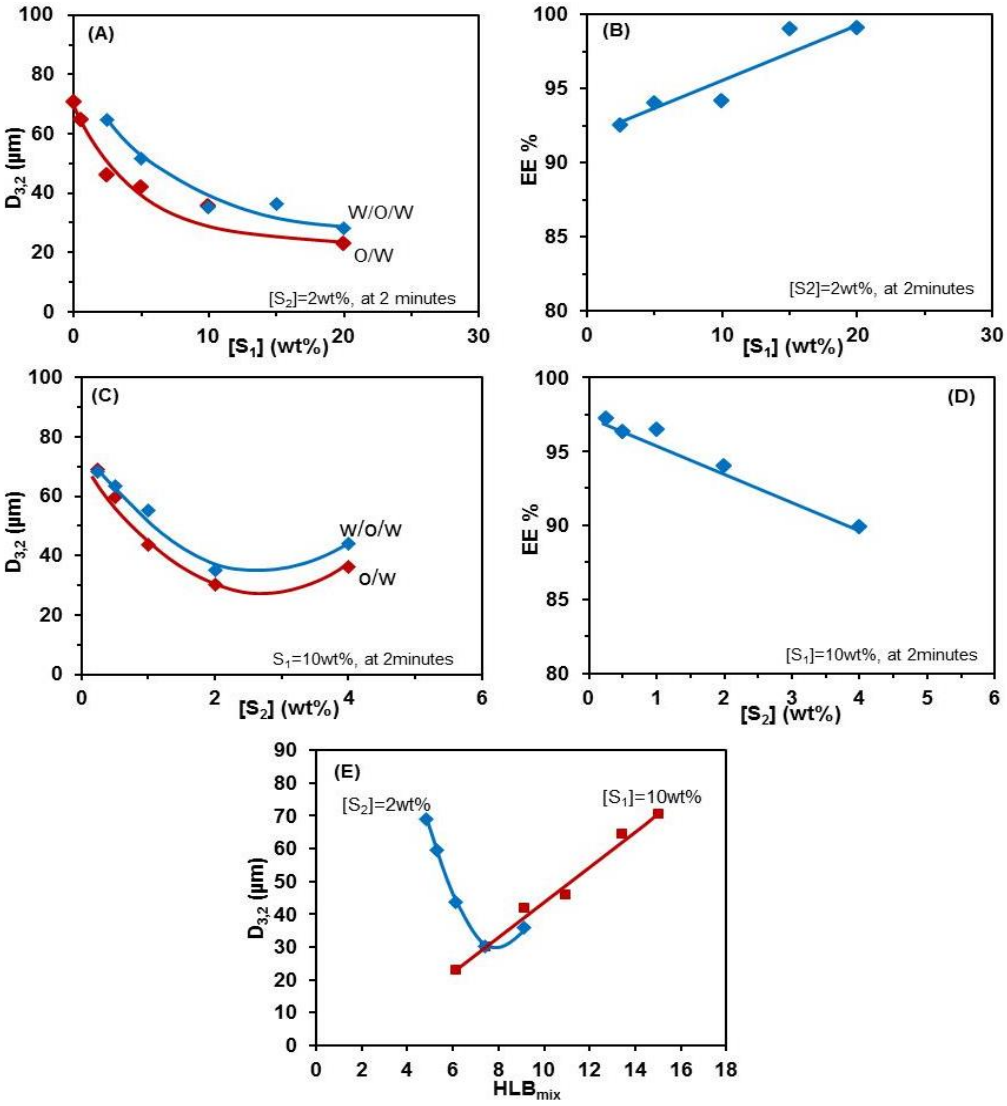


Figure 3.23. (A) $D_{3,2}$ of emulsions; B) Encapsulation efficiency of multiple emulsions versus the concentration of Span 80; C) $D_{3,2}$ of emulsions against the concentration of Tween 80 in the water phase; D) Encapsulation efficiency of multiple emulsions against the concentration of Tween 80 in the water phase (E) $D_{3,2}$ of single emulsions versus HLB of the surfactants, ($\emptyset_{1,0}$ = 0.30, $\emptyset_{2,0}$= 0.33,N_1= 9400 rpm, N_2= 3400 rpm, [S_1] = 2%, [NaCl] = 0.1M in water. (Data of W/O/W in (A) and (C) and data in (B) and (D) from Paramalingam, 2012).

The drop size of single emulsions produced with varying [S_1] and [S_2] versus the HLB of emulsions are presented in Figure 3.23(E). As the concentration of Tween 80 increases, the overall HLB of the emulsion increased, while increasing Span 80 concentration decreased the emulsion HLB. From this figure, and as

previousely mentioned, one can observe that smallest droplets are produced when the multiple emulsions HLB is within the range 7-9 (near the optimum HLB). Multiple emulsions formed with surfactants HLB out of this range have larger drops. This is also associated with the enhanced possibility for an internal water droplet to exit during secondary emulsification, which results in lower encapsulation efficiency/larger release. When the oil droplets are very small, they are less likely to be further ruptured which minimise the NaCl release during the secondary emulsification.

From the data presented in Figure 3.23(E), it can be concluded that the relative concentration of surfactants can play a significant role on encapsulation efficiency, as well as the stability of multiple emulsions. Both surfactant micelles can solubilise the other surfactant. With increasing the concentration of Tween 80 in water and formation of micelles above CMC, some of the oil-soluble surfactants will emigrate into the micelles, therefore increasing HLB further at the interface. Similarly, the water-soluble surfactant can be solubilised in the oil phase. While these effects appear to be more related to long-term stability, they can still be important during emulsification. It has been reported that when the concentration of hydrophilic surfactant in the continuous phase of W/O/W emulsion increases the system becomes less stable (Schmidts et al., 2009). This is because when the concentration of hydrophilic surfactant in the outer phase is higher than its CMC, the hydrophobic surfactant in the primary emulsion might solubilise in the hydrophilic surfactant and move it to the continuous phase. As a result, the concentration of hydrophobic surfactant decreases in the primary emulsion which causes the rupture of oil layer and the release of internal droplets.

3.2.4.4. Primary phase fraction, $\emptyset_{1,0}$

Figure 3.24(A) show encapsulation efficiency of multiple drops versus primary phase fraction $\emptyset_{1,0}$. The results indicate that the encapsulation efficiency of multiple emulsions decreases as primary phase fraction $\emptyset_{1,0}$ increases. It is believed that increasing $\emptyset_{1,0}$ enhances the viscosity of the primary emulsion and makes it more concentrated. Any deformation of compact/concentrated droplets, when exposed to shear stress during secondary emulsification, can force the internal droplets near the interface to exit to the external phase, causing an increase in NaCl release which leads to a reduction in the drop size, see Figure

3.24(B). This figure shows that the drop size of multiple emulsions decreases as $Ø_{1,0}$ increases.

Similar results were obtained by Vladisavljević et al (2014). They found that the release rate was higher for smaller outer droplets, due to higher interfacial area per unit volume, as would be expected for the transfer by molecular diffusion.

Typical micrographs of the multiple emulsions are illustrated in Figure 3.24(C), which shows that the drop size decreases with increasing $Ø_{1,0}$. These micrographs also indicate that the encapsulation efficiency decreases as $Ø_{1,0}$ increases.

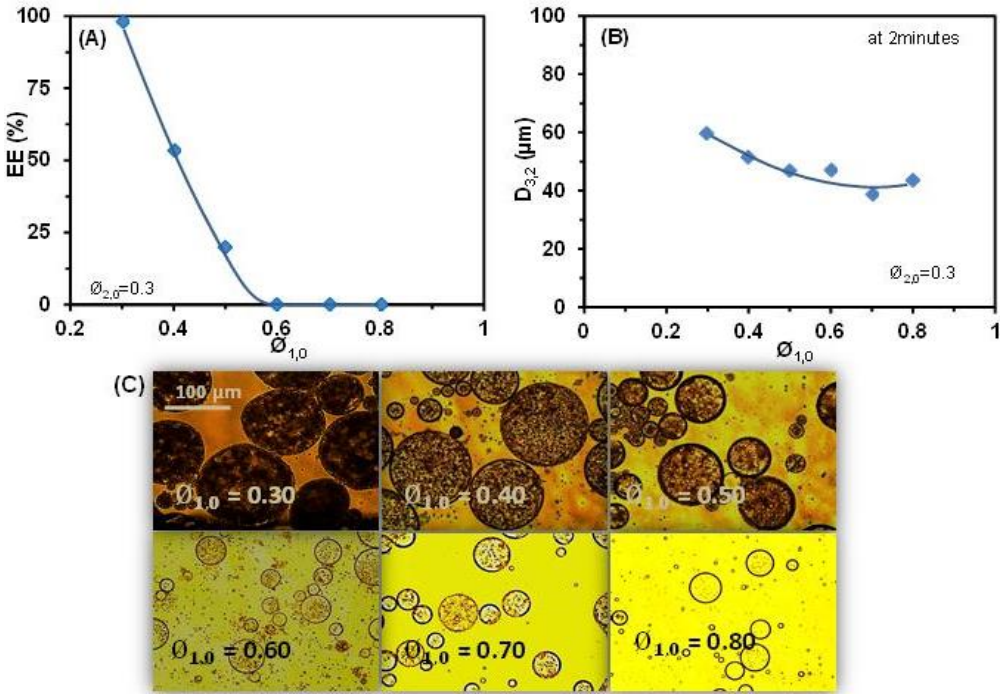


Figure 3.24. A) Encapsulation efficiency of the multiple emulsions, and B) $D_{3,2}$ versus $Ø_{1,0}$, C) Micrographs of multiple emulsions at different $Ø_{1,0}$, ($Ø_{2,0}$= 0.30, N_1= 9400 rpm, N_2= 3400 rpm, [S_1] = 5%, [S_2] = 2%, [NaCl] = 0.1 M).

3.2.4.5. Secondary phase fraction, $Ø_{2,0}$

In this set, various formulations of W/O/W emulsions with different secondary phase fractions $Ø_{2,0}$ were prepared. Figure 3.25(A) shows that encapsulation efficiency of multiple drops increases with $Ø_{2,0}$. The results in Figure 3.25(B) shows the size of multiple drops, $D_{3,2}$, passes through a maximum value with increasing $Ø_{2,0}$. When varying the secondary phase fractions $Ø_{2,0}$ in wide range, we see two well-defined regions:

- At low volume fractions, $Ø_{2,0}$ < 0.4, there is an increase in drop size with $Ø_{2,0}$.

- At higher volume fractions, $Ø_{2,0}$ > 0.4, there is a significant decrease in drop size with increasing $Ø_{2,0}$.

Vankova et al., (2007) observed similar behaviour in their work. They concluded that the drop size decreases significantly with increasing the oil phase fraction above 0.40 for emulsions of mineral oils. They explained this with the transition from inertial regime of emulsification (at low phase fraction) to viscous regime of emulsification (at high phase fraction); due to an increase of the size of the smallest, turbulent eddies in the turbulent flow.

To compare these results with those for single emulsions a series of single O/W emulsions were prepared (at different $Ø_{2,0}$ assuming that all internal water droplets were released) and the results were compared with multiple emulsions see Figure 3.25 (B). It was found that $D_{3,2}$ for single O/W emulsions followed the same trend as $D_{3,2}$ for multiple W/O/W emulsions.

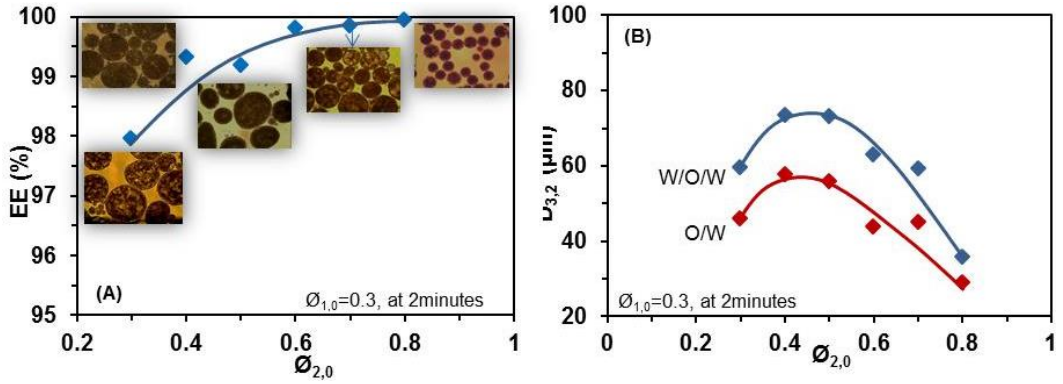


Figure 3.25. A) Encapsulation efficiency and B) $D_{3,2}$ of multiple emulsions versus $Ø_{2,0}$, ($Ø_{1,0}$= 0.30, N_1= 9400 rpm, N_2= 3400 rpm, [S_1] = 5%, [S_2] = 2%, [NaCl] = 0.1 M).

3.2.4.6. Oil phase viscosity

One of the approaches to increase the encapsulation efficiency and stability in multiple emulsions is to increase the oil phase viscosity. The surfactant type and concentration can also contribute to the viscosity of oil phase if they are used at high concentrations, but usually surface active properties are hard to decouple from their viscosity effects. Using a mixture of oils can be used to vary the viscosity of the oil phase to control the movement of the solute across the oil membrane (Sinha and Kumar, 2002).The viscosity of the middle phase is mainly affected by the oil type and composition. To explore the effect of oil composition on encapsulation efficiency of W/O/W multiple emulsions, paraffin oil was mixed with cyclohexane at different ratios and used as the oil phase. The viscosities

(weight-average viscosity) of the oil mixtures and the resulting are presented in Figure 3.26(A). It is obvious that increasing the amount of the paraffin oil in the oil mixture the overall viscosity of the oil mixture increases. The viscosity of resulting multiple emulsions decreases as the amount of paraffin oil in oil phase increase; see Figure 3.26(A).

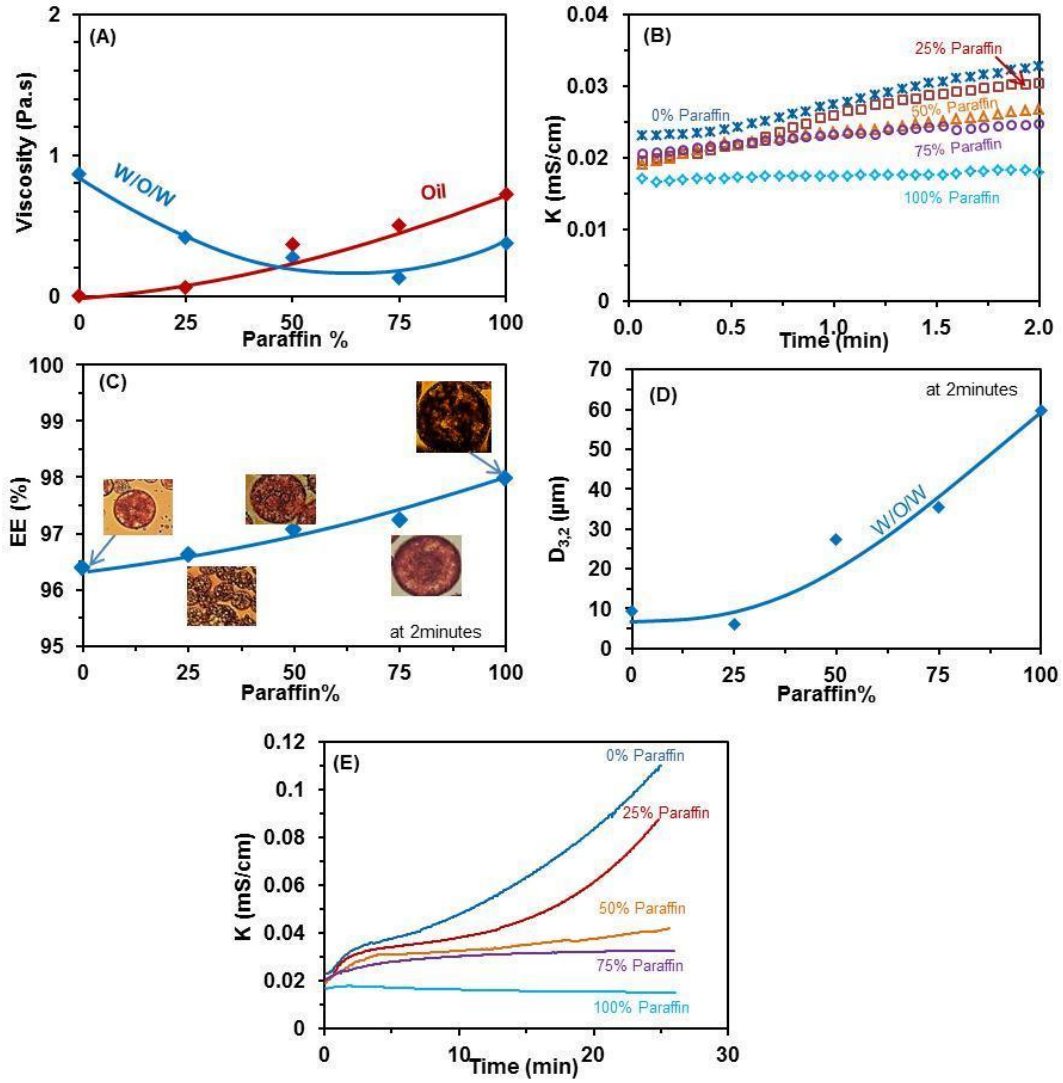


Figure 3.26. (A) Viscosity of the oil phase and multiple emulsions versus paraffin concentration, B) Conductivity of multiple emulsions during secondary emulsification versus time at different paraffin concentration, C) Encapsulation efficiency of multiple emulsions versus paraffin concentration, D) $D_{3,2}$ of multiple emulsions versus paraffin concentration in the oil phase, E) Conductivity of multiple emulsions during and after emulsification versus time at different paraffin concentration, ($Ø_{1,0}$= 0.30, $Ø_{2,0}$= 0.30, N_1= 9400 rpm, N_2= 3400 rpm, [S1] = 5%, [S2] = 2%, [NaCl] = 0.1 M).

Figure 3.26(B) shows conductivity of multiple emulsions during secondary emulsification at different oil phase paraffin compositions. It can be seen that decreasing oil viscosity (by adding cyclohexane to paraffin oil) leads to an increase in conductivity during secondary emulsification, which means more

release of inner droplets as shown in Figure 3.26(C) in terms of encapsulation efficiency. Although the encapsulation efficiency was already high (just above 96%) when pure cyclohexane is used as the oil phase, but adding highly-viscous paraffin oil to cyclohexane further improved the encapsulation efficiency by increasing the oil-phase viscosity. The reason is mainly due to resistance against diffusion of salt (i.e., inner droplets) through the viscous oil membrane. As expected a higher viscosity of the oil phase prevents water droplet from expulsion to the external water phase.

The micrographs shown in Figure 3.26(C) proves the existence of highly-loaded multiple drops at high oil-phase paraffin compositions. These results are in the good agreement with what Bonnet *et al*. found in 2009. It seems that at the base conditions of the experiments in this study (which have been optimised) the oil-phase viscosity does not play an important role in release of the inner phase as other variables control the rate of release.

However, the viscosity of the oil phase can significantly affect the drop size of multiple emulsions as seen in Figure 3.26(D). This figure shows that the drop size increases as the oil viscosity increases. This is because the drop breakup process is much less affected by the oil viscosity (Vankova et al., 2007). It is clear that at higher oil-phase paraffin compositions the drops could maintain the inner droplets and so remain larger, while the drops size at lower oil-phase paraffin compositions are smaller due to release of inner droplets. From a post-emulsification stability study it is clear that the highly-viscous oil phase maintain a significantly higher stability as shown in Figure 3.26(E).

3.2.5. General conclusion

Inclusion can play an important role during preparation of multiple emulsions when a very high lipophilic surfactant or very low primary phase fraction is used. Under the conditions of this study, it was found that inclusion did not occur significantly during preparation of multiple emulsions.

It is possible to outline an optimum formulation for preparation of stable multiple emulsions with high encapsulation efficiency. In general, the optimum conditions include higher stirring speed and surfactant concentration, and lower dispersed phase fraction in primary emulsification stage in comparison to the secondary emulsification stage.

3.3. Alginate hydrogel particles

3.3.1. Introducing the ideas

Alginate hydrogels have many applications in different areas especially in pharmaceutical and biomedical industries due to their biocompatibility and non-toxicity.

One of the common methods to produce alginate particles is emulsification/gelation method. In this method alginate hydrogel particles are synthesised by dropwise addition of $CaCl_2$ solution to emulsion of an alginate aqueous solution in oil. However, particle size distribution is wide due to random coalescence of drops and particle size cannot be easily controlled, as particles tend to coalesce with each other before hardening properly.

Size of the pores and their interconnectivity have an important impact on the encapsulation efficiency and release of the active agent from the polymer matrix, as well as the mechanical properties of the hydrogel. Large pore sizes are advantageous for applications in tissue engineering, or where a large water content is required.

In this work, the initial idea was to make macroporous alginate hydrogel particles via CPI and through inclusion of an oil to polymerising particles. To see the possibility of inclusion we carried out simple experiments. NaAlg aqueous solution was emulsified in different oils (octane, cyclohexane, and vegetable oil) and the conductivity of the emulsion was monitored with time. The NaAlg solution contained a water-soluble surfactant to improve and facilitate the inclusion of the oil phase.

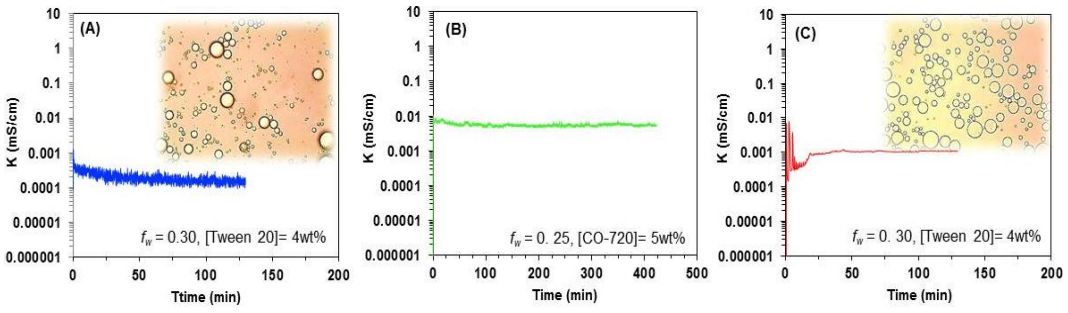


Figure 3.27. Conductivity versus time for CPI (SW); A) Octane, B) Cyclohexane, C) Vegetable oil ([NaAlg]= 1wt%, Stirring speed= 500 rpm, T=25°C).

As seen in Figure 3.27 the conductivity of the emulsions did not change significantly and specially did not increase at all, for at least 2 hours after the start of the emulsification, indicating no inclusion of the oil phase. A reason for no

inclusion could be due to relatively high viscosity of NaAlg solution (75 mPa.s) in comparison to that of cyclohexane (0.88 mPa.s) and octane (0.53 mPa.s). The higher viscosity of drops may prevent or at least significantly retard the inclusion of oil phase to the drops. Microscope micrographs also did not reveal any multiple drops. Vegetable oil has a higher viscosity (0.49 Pa.s) than alginate solution but, however, we did not detect any inclusion. The next idea was to carry out a feasibility study on a new emulsification method, which is a modification of the current emulsification technique (Chan et al., 2000; Zhang et al., 2006; Ribeiro et al., 2005). In this method $CaCl_2$ is also emulsified in oil, using a homogeniser to produces micron-size drops, before it is mixed with the NaAlg emulsion. In fact, two W/O emulsions of $CaCl_2$ and NaAlg are mixed to synthesise macroporous alginate hydrogel particles. The modified method increases the chance of coalescence of NaAlg and $CaCl_2$ drops and could narrows the hydrogel particle size distribution.

3.3.2. New method of synthesis of alginate hydrogel particles

The proposed method is based on coalescence between NaAlg and $CaCl_2$ drops, in a continuous oil phase. It is expected that as soon as drops of $CaCl_2$ and NaAlg collide, crosslinking at the interface of the drops leads to formation of a film of water-insoluble polymer (CaAlg), which could prevent further coalescence. Although $CaCl_2$ molecules could still diffuse into NaAlg drops and so crosslinking reactions continue inside NaAlg drops to form polymer matrix. But as drops are exposed to continuous coalescence, large irregular-shape macroporous polymer particles are anticipated to form.

In the following sections, we present and discuss the results of a preliminary study on synthesis of alginate hydrogel particles via mixing the W/O emulsions of NaAlg and $CaCl_2$. Overall the results are somehow promising, and so recommendations have been made for future work on this area.

3.3.3. Synthesis of hydrogel particles

Based on the new method introduced above, a two-step method is proposed in order to synthesise macroporous alginate hydrogel particles. In the first step, two normal W/O emulsions of NaAlg and $CaCl_2$ aqueous solutions in vegetable oil (containing Span 20) are prepared. In the second step, the two emulsions are mixed at 500 rpm for 2 hours, while samples are frequently taken for optical

microscopy analysis. The produced semi-solid hydrogel particles are then washed and dried for SEM analysis. The schematic of the method is illustrated in Figure 3.28. (Also see Chapter 2 for more details of the methodology.)

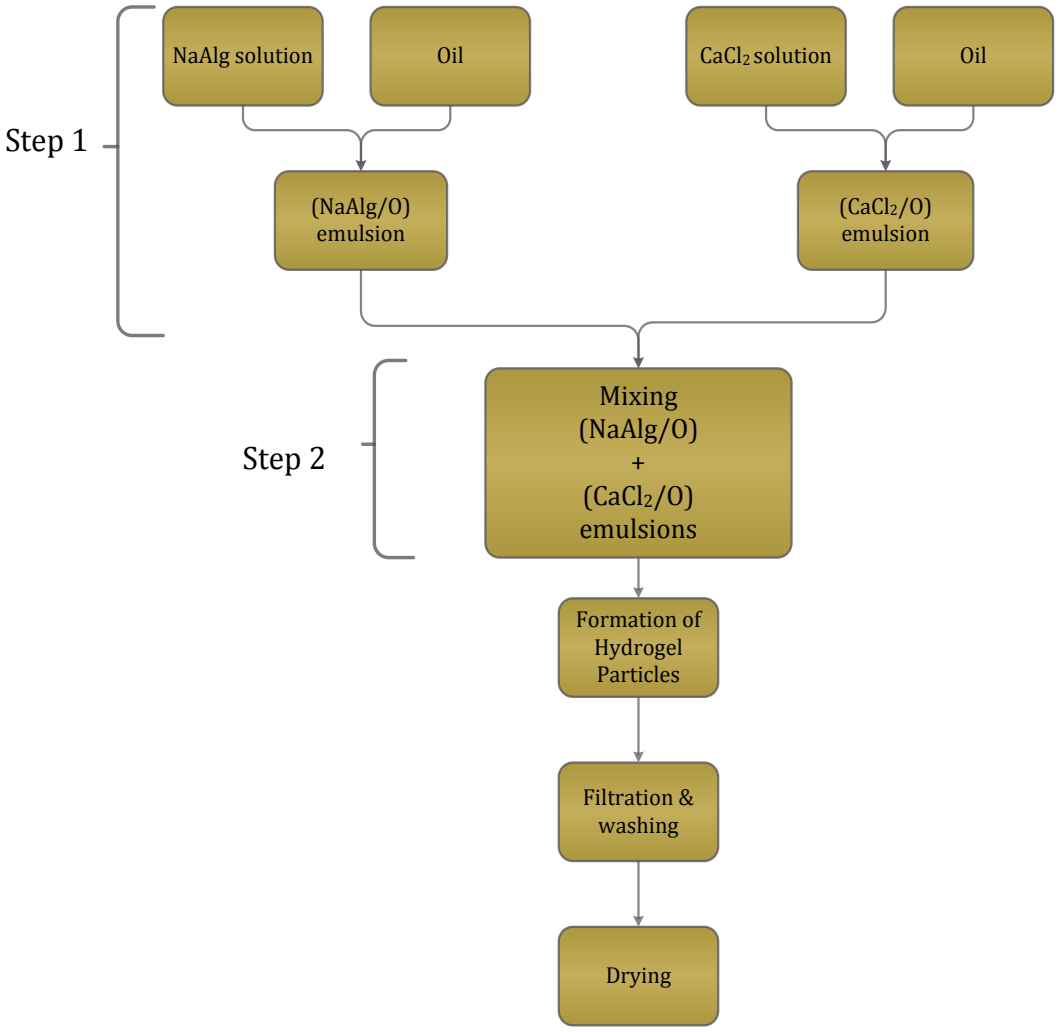


Figure 3.28. Illustration of the proposed two-step method for synthesis of hydrogel particles.

This preliminary study look at the effects of NaAlg and $CaCl_2$ concentrations on the size and morphology of the polymer particles. Table 3.3 shows the formulations used for this study. Before discussing the results of these experiments, it is helpful to look at the results of the base experiment (experiment 1 in Table 3.3). Figure 3.29 (A) and (B) show micrographs of the initial NaAlg and $CaCl_2$ emulsions, respectively. The average drop size of NaAlg and $CaCl_2$ drops are 7 µm and 1 µm, respectively. The formation of polymer particles with time after mixing the two emulsions are also shown in Figure 3.29(C) to (G). Interestingly, large multiple drops can be seen after just 10 min of the start of the

mixing. Soft particles are visibly formed after 45 min of mixing and grow with time. However, stable semi-solid hydrogel particles (which can be washed and dried without loss) are formed after around two hours of emulsification (Figure 3.29(G)) with average diameter of 110 µm. Each large particle seems to contain small gel like domains and voids (water droplets) dispersed in a continuous polymer matrix. The dried particles shown in Figure 3.29(H) have an average size of 20 µm.

Table 3.3. Different formulations to prepare hydrogel particles.

Experiment number	NaAlg Emulsion				$CaCl_2$ Emulsion, f_w 25%		
	[NaAlg] (%)	[S] (%)	f_w (%)	N_1(rpm)	[$CaCl_2$] (%)	[S] (%)	N_2(rpm)
1	1	0.3	25	500	3	0.3	3200
2	1.5	0.3	25	500	3	0.3	3200
3	2	0.3	25	500	3	0.3	3200
4	4	0.3	25	500	3	0.3	3200
5	1	0.3	25	500	6	0.3	3200
6	1	0.3	25	500	1	0.3	3200

S : Span 20

f_w : Phase fraction.

N_1: Stirring speed for NaAlg emulsion.

N_2: Stirring speed for $CaCl_2$ emulsion.

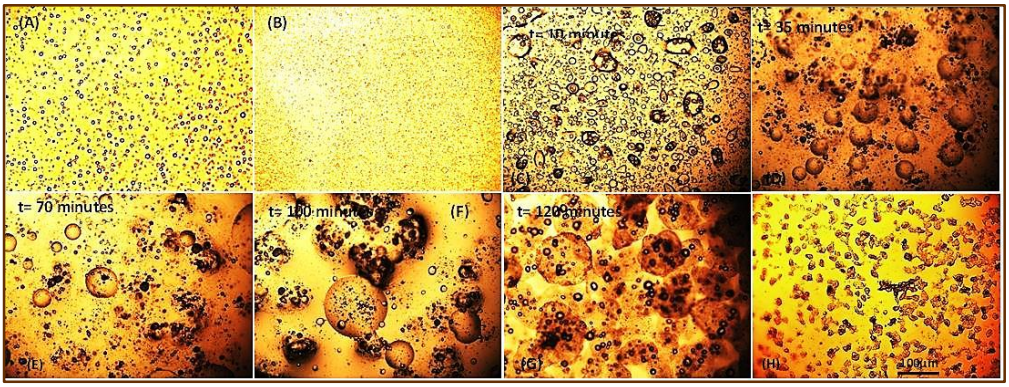


Figure 3.29. Optical micrographs for initial (A) NaAlg emulsion, (B) $CaCl_2$ emulsion, (C, D, E, F, G, H) drops/particles at different time after mixing the emulsions, (Experiment 1, see Table 3.3).

Based on the observations a mechanism suggested for formation of these large hydrogel particles, which is demonstrated in Figure 3.30. As soon as a $CaCl_2$ drop colides with a NaAlg drop, polymer is formed at the interface of the drops and forms a film preventing further mixing of the drops. But small molecules of $CaCl_2$ can still diffuse through the polymer film interface and react with NaAlg. This process is repeated every time a 'small' drop of $CaCl_2$ collides with a 'big'

NaAlg drop. These formed structures may coalesce with each other and develop large irregular-shape polymer matrices with large voids (pores) inside them.
In the following sections a brief account of the effects of some parameters on the size and morphology of polymer particles are presented and discussed.

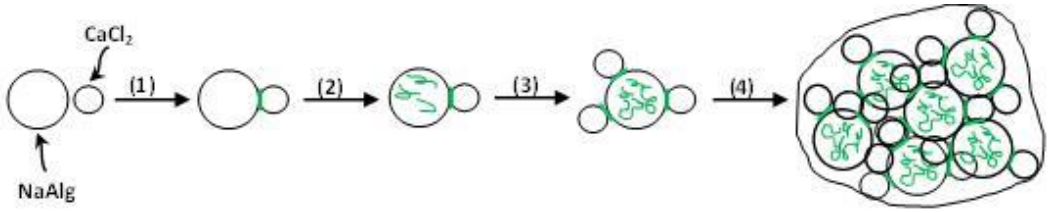


Figure 3.30. Mechanism of formation of large polymer matrices with voids via coalescence of NaAlg and $CaCl_2$ drops (Not to scale); (1) coalescence and immediate formation of polymer at interface of drops, (2) diffusion of CaCl2 into NaAlg drops and formation of polymer, (3) coalescence of more $CaCl_2$ drops to polymerising NaAlg drops, (4) coalescence of polymerising drops and formation of large hydrogel particles with voids (very large pores).

In order to validate the suggested mechanism, a simple experiment was carried out. A drop of NaAlg solution and a drop of $CaCl_2$ solution was placed close to each other on a glass slide and their behaviour was monitored for an hour under optical microscope. In order to distinguish between the drops and study the mixing of the drops, a dye was added to the $CaCl_2$ solution to make it reddish.

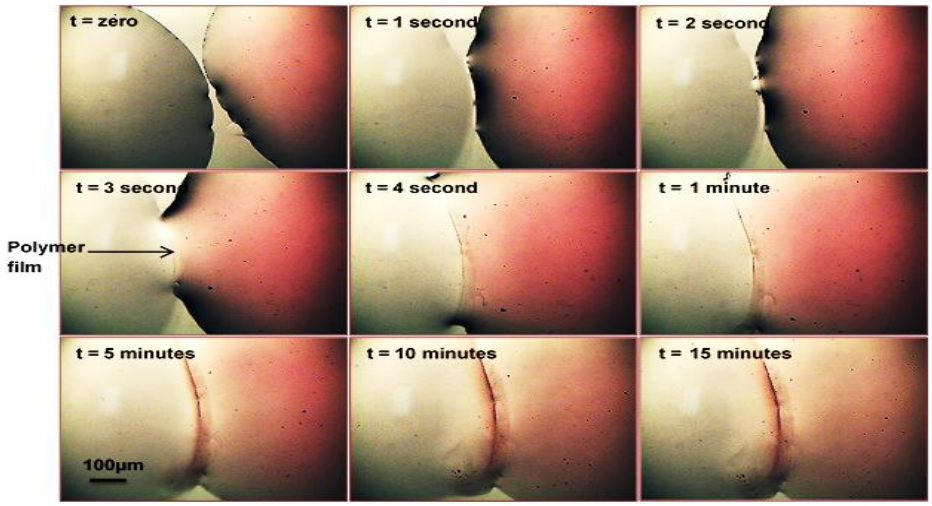


Figure 3.31. Micrographs of a NaAlg drop and a dyed $CaCl_2$ drop touching at the surface of a glass slide. Development of the polymer film at the interface of the drops is shown with time.

Figure 3.31 shows some of the micrographs of the interface of the drops with time. Interestingly, just after a few seconds of drops touching each other, a polymer film is formed which prevented further mixing of the drops. The film becomes thicker with time and the NaAlg drop becomes visually like a gel (i.e., a soft particle) after about 15 minutes. This simple experiment shows that the

suggested mechanism is a valid explanation of the formation of large hydrogel particles.

Here the results of a brief study on the effects of two of the most important parameters, concentration of NaAlg and $CaCl_2$, are presented and discussed.

Sodium alginate concentration

As seen in Table 3.3 experiments 1, 2, 3 and 4 were conducted to investigate the effect of NaAlg concentration (1-4 wt%) on the formation of hydrogel particles. It is worth mentioning that we didn't go above 4 wt% of sodium alginate. The NaAlg solution above this value becomes very viscous and cannot be handled easily.

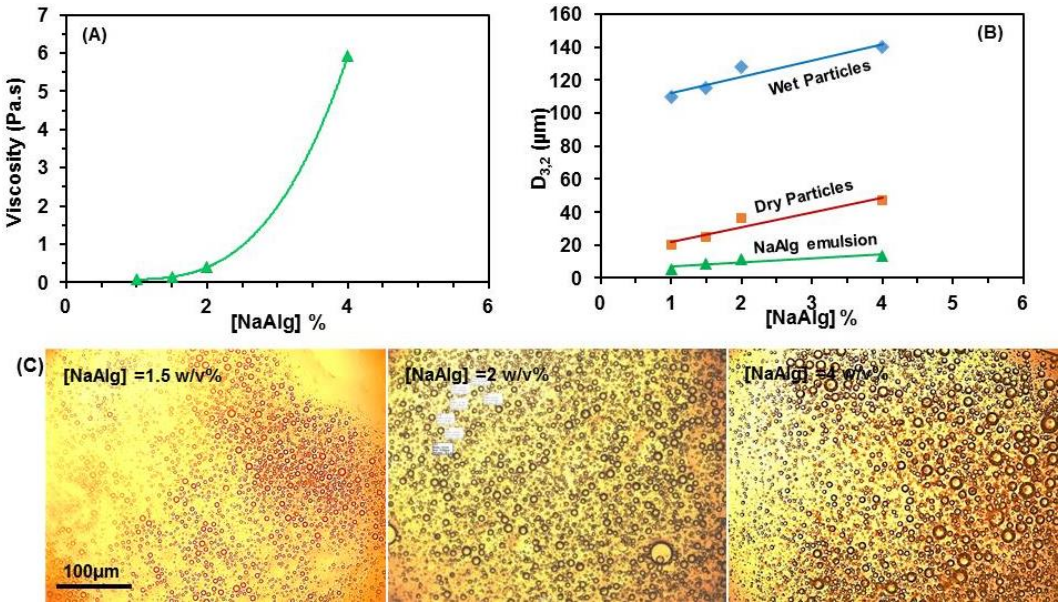


Figure 3.32. Variation in (A) viscosity of the solution; (B) average drop size of initial NaAlg drops, wet and dry hydrogel particles; with NaAlg concentration. (C) Micrographs of NaAlg emulsions with 1.5 (far left), 2, and 4 wt% NaAlg.

Figure 3.32(A) shows the effect of the NaAlg concentration on viscosity of its aqueous solution. As the viscosity of the NaAlg solution increases the drop size of the emulsion increases as well, see Figure 3.32(B), due to increase in viscous forces. The micrographs (Figure 3.32(C)) supported the increase in drop size. Increasing the concentration of NaAlg shows a significant increase in the size of polymer particles formed after 2 hours mixing with $CaCl_2$ emulsion, as seen in Figure 3.32(B). This is due to the initial larger NaAlg drops being the locus of crosslinking reactions, which, in turn, leads to formation of larger polymer matrices.

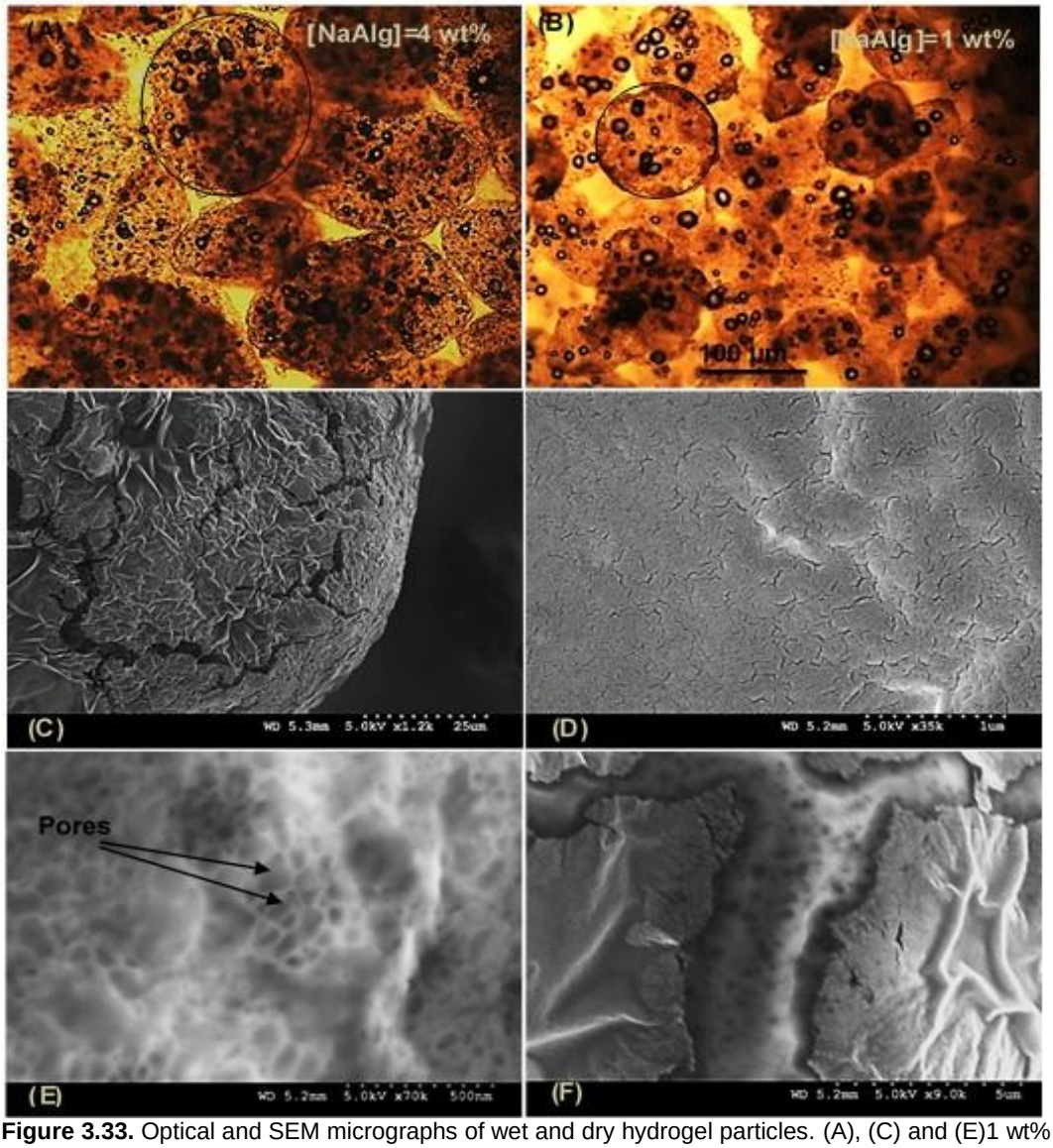


Figure 3.33. Optical and SEM micrographs of wet and dry hydrogel particles. (A), (C) and (E)1 wt% NaAlg; (B), (D) and (F) 4 wt% NaAlg. (C)-(F) SEM micrographs of surface structure of particles; Note the scale bars for SEM micrographs, which are different.

Micrographs obtained using optical microscope (wet particles) and SEM (dry particles) are shown in Figure 3.33(A)-(D). It has been reported that higher NaAlg concentration decreases the rate of gelation of the interfacial film as the ratio of NaAlg to $CaCl_2$ increases (Blandino et al., 1999). As a result of this, diffusion of $CaCl_2$ through the polymer film is facilitated and polymer with lower crosslink density is formed. This allows 'sticky' polymerising (gelling) alginate particles to coalesce with each other to form larger structures. The porous structure of the hydrogel particles can be seen in SEM micrographs in Figure 3.33(E)-(H). The pores (the dark spots) resulted from the removal of the trapped water in the crosslinked polymer matrix during the washing/drying process. The average pore

size seems to be below 1 μm. However, we could not take images from the internal structure of the particles.

Calcium chloride concentration

Experiments 5 and 6 were conducted at different $CaCl_2$ concentrations of 6 wt% and 1 wt%, respectively. The $CaCl_2$ concentration range used here does not affect its emulsion drop size as it does not have a significant effect on viscosity or surface tension of the solution. The average $CaCl_2$ drop size is around 1 μm for all $CaCl_2$ concentrations used (1, 3 and 6 wt%).

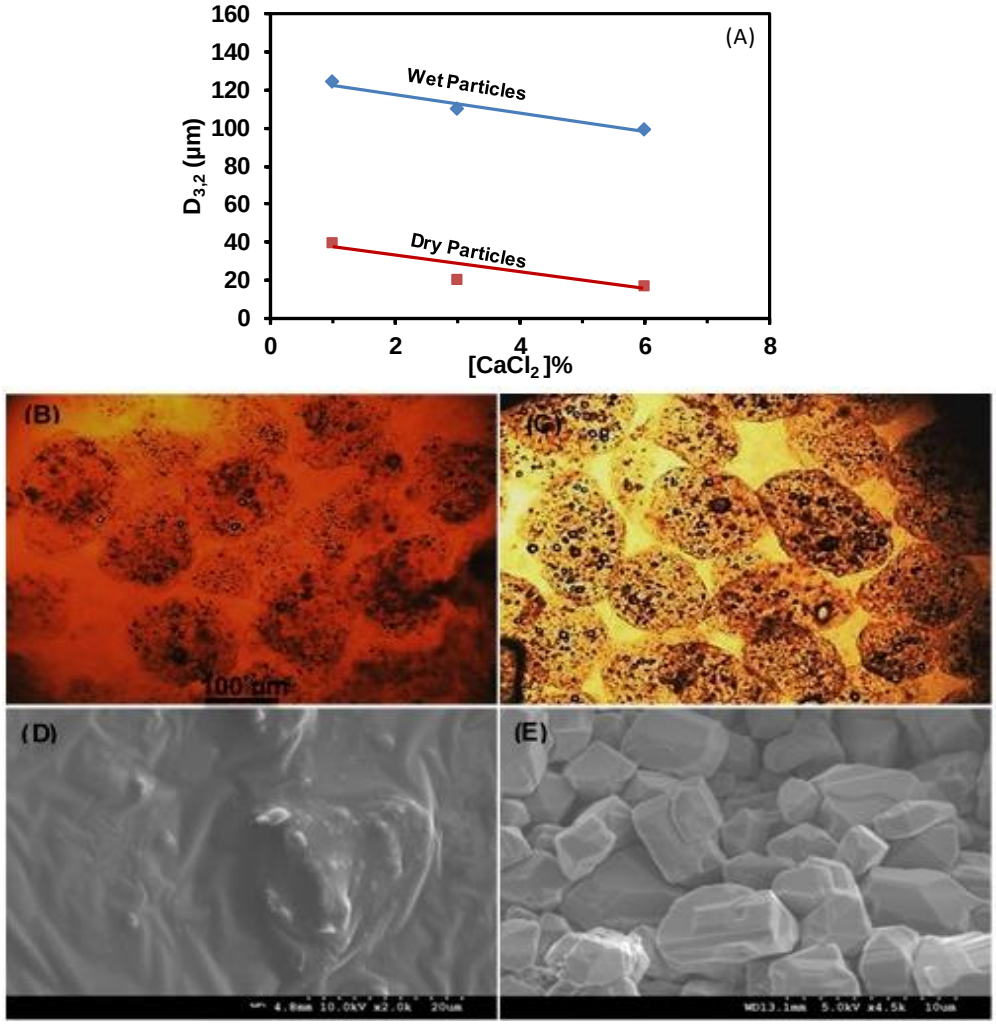


Figure 3.34. (A) Variation in average drop size of wet and dry hydrogel particles with $CaCl_2$ concentration. (B) and (C) Optical micrographs of wet hydrogel particles; (D) and (E) SEM micrographs of a typical hydrogel particles for the effect of $CaCl_2$ concentration for [$CaCl_2$] =6 and 1%, respectively.

Figure 3.34(A) shows average size of the hydrogel particles decreasing as the concentration of $CaCl_2$ increases. Optical microscopic images of the wet hydrogel

particles and SEM micrographs of dry ones are seen in Figure 3.34(B)-(E). Particles formed at 6wt% $CaCl_2$ were too soft and agglomerated during washing and drying, which made it very difficult to measure their size. They also melted while trying to analyse them using SEM (see Figure 3.34(D)). Increasing $CaCl_2$ concentration results in immediate formation of thicker and more crosslinked polymer film at the interface of the drops and decrease in flux of $CaCl_2$ through the polymer membrane. This has also been reported in literature that $CaCl_2$ concentrations above 4 wt% leads to significant increase in diffusion resistance of $CaCl_2$ movement through the gel film (Blandino et al., 1999). This can explain lack of crosslinking reactions inside NaAlg drops, which leads dissolving (mostly hydrophilic NaAlg) polymer through washing process and melting during SEM analysis. Lower $CaCl_2$ concentrations is equivalent to higher NaAlg concentration and so, as described above, polymer with lower crosslink density is formed, which are more susceptible to coalescence with each other and form larger structures. Mokhtari et al. (2017) reported that size of alginate nanoparticles, prepared by emulsification method, decreases as higher molarity of $CaCl_2$ and lower alginate concentrations are used.

Particle size distribution

Interestingly, The size distribution of produced particles is quite narrow as shown in Figure 3.35. In fact, the size distributions for all experiments carried out in this study are relatively narrower in comparison to those reported in literature (Poncelet et al., 1992, 1995; Mukhtari et al., 2017).

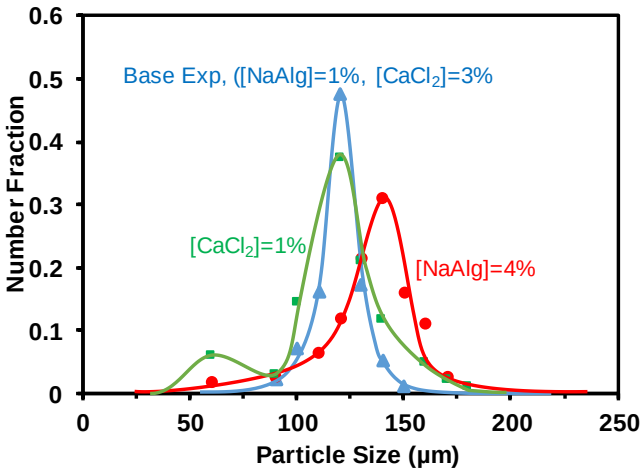


Figure 3.35. Particle size distributions for the base experiment ([NaAlg]=1%, [$CaCl_2$]=3%), and experiments with different NaAlg and $CaCl_2$ concentrations

Although, the coalescence of gelling particles is a random process, but the extent of the coalescence seems to happen in a controlled and uniform manner leading to formation of uniform particles in terms of size. This can be taken as an advantage over the current emulsification technique used for producing alginate particles

Swelling of synthesised hydrogel particles

Alginate gels have the ability to increase in size under different conditions (Ching et al., 2017). Moe et al. (1993) showed that dried alginate beads swell immediately to their original size when rehydrated in water.

In this research, the swelling of the synthesised hydrogel particles was determined by measuring the increase in size of dry particles after immersing them in water until they reach the equilibrium state. Figure 3.36(A) shows a sample of hydrogel particles in dry and swelled states and Figure 3.36(B and C) demonstrates the swelling of the particles obtained with different NaAlg and $CaCl_2$ concentrations respectively.

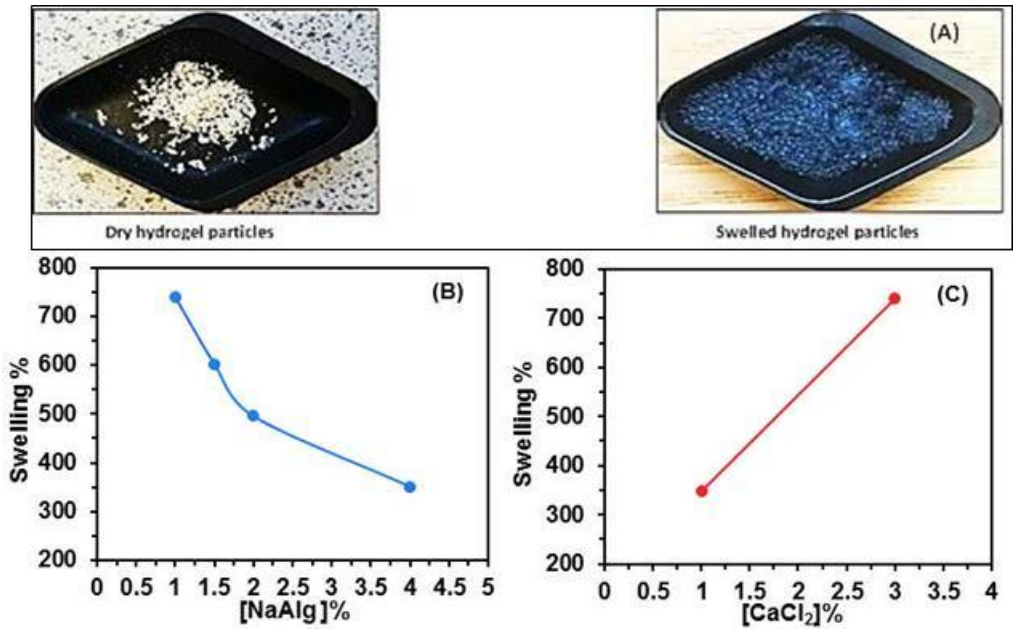


Figure 3.36. (A) Images of dry and swollen samples of hydrogel particles (experiment 1). (B) Swelling of hydrogel particles obtained at different NaAlg concentrations for experiments 1, 2, 3 and 4. (C) Swelling of hydrogel particles obtained at different $CaCl_2$ concentrations for experiments 1 and 3, (Swelling %=(wet weight - dry weight)/dry weight×100).

The swelling is between 350%-740%, the highest value (740%) belonging to the base experiment. It was reported in literature the maximum range for alginate is (250%) (Bajpai and Kirar, 2016). Swelling of particles increases with increasing

$CaCl_2$ concentration and decreasing NaAlg concentration; matching the conditions under which smaller particles are formed, see Figure 3.36(B) and (C). These figures reveals that intermediate $CaCl_2$ concentration (around 3wt%) and low NaAlg concentration (the base experiment) are close to 'optimum' conditions to achieve the highest absorption capacity (740% in mass basis). This is also in agreement with literature (Mokhtari et al. 2017). However, further investigation is required to consolidate the claims made by this preliminary study.

3.3.4. General Conclusion

The modified two-step method to synthesise alginate hydrogel particles produced some promising results; micron-size particles with narrow size distribution. However, the method should be further studied in order to outline the optimum conditions for production of alginate hydrogel particles. See Chapter 4 for recommendations for future work.

Chapter 4

CONCLUSIONS AND RECOMMENDATIONS

This chapter summarises the main conclusions drawn from the findings and demonstrates some recommendations for future work.

4.1. Conclusions

❖ CPI Emulsification

A comprehensive study of inclusion in catastrophic phase inversion (CPI) was carried out in this work for the first time. The aim was to study the inclusion as underlying mechanism of CPI and its effect on dynamic of phase inversion and final drop size. The objectives were to study the effects of important formulation and process variables on the rate and extent of inclusion, inversion time, and final drop size.

Another objective of this study was to compare the CPI and direct emulsification methods with respect to their capability to produce fine emulsions.

The conclusions are drawn below, showing that the objectives of the study have been fulfilled, followed by the recommendations for future work.

Inclusion and superiority of CPI over direct emulsification method

Inclusion is the core mechanism of inversion of emulsions via CPI emulsification. Process and formulation variables affect the extent and rate of the inclusion as itemised below. In most cases, the more inclusion the longer the inversion time and the smaller the final drop size is in inverted emulsion.

CPI emulsification is a superior method in comparison to direct emulsification for producing fine emulsions, mainly when the inclusion extensively happens leading to formation of smaller inner droplets, which in turn form the dispersed phase in the inverted emulsion. Below the effect of each parameter is

i. Water volume fraction (f_w): Rate of inclusion was faster at higher f_w. Both inversion time and extent of inclusion reduced as the water volume fraction in the initial abnormal W/O emulsion increases. Inclusion decrease as f_w increases when single (HLB=14) or mixed surfactants is used. However, inclusion occurs more extensively at higher HLB of 14.

ii. Surfactant affinity (HLB): Extent of inclusion increases as HLB of the surfactant(s) increases. For both direct and CPI methods, the Sauter mean diameter $D_{3,2}$ of the drops increases as HLB of the surfactant increases. The difference between direct and CPI methods with regard to droplet size is more significant at higher HLBs than the lower HLBs.

iii. Stirring speed: Rate of inclusion is faster as stirring speed decreases and it is greater when the surfactant is dissolved in the oil phase. It was found that inversion time and drop size are strongly affected by stirring speeds. At high HLB of 14, the inversion time initially increases with rpm, but later decreases, showing a maximum in the intermediate range. In contrast, for a mixture of surfactants (HLB=13), the inversion time and drop size decreased with increasing stirring speeds.

Overall, placing surfactant in oil phase produces finer emulsions than water phase via CPI method. This is because when the surfactant dissolved in the oil phase requires more time to migrate from the oil phase to the water phase.

In comparison to direct method, CPI is the superior method as it can produce finer emulsions at low stirring speeds, i.e. less energy input. However, at very high stirring speeds the drop sizes of the produced emulsion converge which indicates when the rate of inclusion is very fast (i.e., short inversion time) the superiority of CPI diminishes.

iv. Temperature: Extent of inclusion decreases as temperature increases. CPI is more efficient in producing finer drops at high temperatures than at low temperatures, with a shorter time for the emulsion to invert. This is due to the increase in the hydrophobicity of the surfactants, which in turn decreases the interfacial tension and favours more break-ups.

In comparison to direct, CPI produces finer emulsions at low temperatures. However, at high value the effect of the temperatures on the drop sizes is less.

- **Multiple Emulsions**

The aim of the study of multiple emulsions was,forthe first time, to investigate the dominant mechanism of release (inclusion vs. other mechanisms) during the preparation of multiple emulsions and the optimum conditions for maximum encapsulation efficiency in freshly-prepared multiple emulsions.

The objectives were to study the effects of different formulation/process variables on encapsulation efficiency and (multiple) drop size in freshly-prepared multiple emulsions. Below the main conclusions are drawn showing that the objectives of this study have been fulfilled. Recommendation have been made for future work.

Dominant mechanism of release during emulsification

i. It was shown that the release mechanism based on diffusion via reversed micelles occurs at long time scales in comparison to mixing time used in this study for secondary emulsification (2 min).
ii. Release via coalescence or deformation of drops, i.e. 'exit' mechanism, appears to be more important during emulsification when drops are exposed to high shear and are deformed so that the internal droplets expelled from the drop.
iii. Osmosis-driven swelling of drops also shown to happen over a period of time and so this instability mechanism can be safely ignored in the course of preparation of multiple emulsions.
iv. The droplet inclusion combined with possible coalescence with other internal droplets inside the oil drop and droplet exit make tracking of release complicated. In order to directly relate the changes in conductivity to release of inner phase, we considered the conditions (surfactant and salt concentration, dispersed phase fraction, etc.) under which inclusion is not important. Therefore droplet exit process is considered as the dominant release mechanism.

Mathematical modelling

i. We derived a modified Brugmann's law for multiple emulsions, which successfully predicts the encapsulation efficiency from the conductivity data.
ii. The derived correlation was validated by considering 100% release and comparing the theoretical and experimental results (obtained for single emulsions), which were in excellent agreement.

Optimum conditions for maximum encapsulation efficiency during emulsification

i. It was found that encapsulation efficiency increases as the stirring speed of the primary emulsification increases.
ii. Using low amount of surfactants is an ideal choice to produce multiple emulsions with high encapsulation efficiency.

iii. It was also found that the drop size increases by decreasing the primary phase fraction, with and without the presence of NaCl.

iv. Encapsulation efficiency was found to be affected by the concentration of the NaCl, encapsulation efficiency increases as NaCl concentration increases. 0.1M of NaCl gives maximum encapsulation efficiency and bigger drop size.

v. A maximum drop size was obtained when intermediate values of secondary phase fraction were used.

vi. Encapsulation efficiency was improved by increasing the viscosity of the oil phase.

❖ **Synthesis of hydrogel particles**

A new two-step method based on coalescence is attempted to produce alginate hydrogel particles. Initial results show that large polymer matrices are formed through coalescence of NaAlg and $CaCl_2$ drops in a W/O emulsion. A mechanism is suggested based on the observations; (i) collision of NaAlg and $CaCl_2$ drops and immediate formation of polymer gel at interface of drops, (ii) diffusion of $CaCl_2$ into NaAlg drops for crosslinking reactions to occur, (iii) steps (i) and (ii) repeats as more small $CaCl_2$ drops collide with the same NaAlg drop, (iv) coalescence of polymerising (gelling) NaAlg drops and formation of large hydrogel structures.

At high $CaCl_2$ concentrations, a thick and dense (highly crosslinked) polymer film is immediately produced and so polymer particles agglomerated upon washing/drying process. It was found that lower NaAlg and intermediate $CaCl_2$ concentrations produced stable particles and high water absorption. The optimum concentrations found to be 1 wt% NaAlg and 3% $CaCl_2$.

The particle size distribution was narrow for all conditions studied here, which could be due to a ‘controlled’ coalescence happening between the gelling particles. This is an advantage of this method over current emulsification method (reported in literature), which lead to formation of wide particle size distributions.

It should be added here that this study was brief, and a thorough investigation needed to fully validate the results and claims made here.

4.2. Recommendations for future works

❖ CPI Emulsification

The following recommendations are suggested for future work:

i. In this study, it was not possible to capture the unstable multiple drops during phase inversion. Study of inclusion can be extended using viscous oils in order to capture the unstable multiple drops in abnormal O/W/O emulsions and visualise the extent and rate of inclusion at different conditions.

ii. The effect of the rate of addition of dispersed phase (water phase) on inclusion and drop size of the inverted emulsion is an important variable that can be studies.

iii. Further study of the effect of stirring speed and temperature would be helpful to fully understand the reasons behind the existence of minima/maxima in inversion time.

iv. Obviously, the type of the oil and the surfactants affect the inclusion, stability, and drop size of the inverted emulsion. These parameters can be investigated considering a specific application.

❖ Multiple Emulsions

The following recommendations are suggested for future work:

i. In order to further study the inclusion in multiple emulsions, one may add a dark dye in the inner aquous phase and monitor the morphology of multiple drops (using an optical microscope) during secondary emulsification. Inclusion of external water (with no dye), extent of coalesence between dyed and non-dyed inner droplets (by monitoring the shade of the internal droplets), and exit of dyed droplets (by monitoring the external phase colour), could be investigated more quantitatively.

ii. The long-term (post-emulsification) stability was not a part of this study, although more stable freshly-prepared multiple emulsions means higher long-term stability as well. A study of long-term stability would be valuable to extent and apply the results of this work to application-oriented research.

iii. The type of the oil and the surfactants affect the mechanism of release, stability, and drop size of the multiple emulsion. These parameters can be investigated considering a specific application.

❖ **Synthesis of hydrogel particles**

i. An attempt to find an oil with inclusion into NaAlg drops was unsuccessful. It is worth to try other types of oil at different conditions to see if inclusion happens. In the presence of inclusion, the synthesised hydrogel particles may have very large porous (voids) with larger water absorption.

ii. A preliminary study of a new method for synthesis of porous hydrogel particles based on addition of $CaCl_2$ W/O emulsion to NaAlg W/O emulsion) proved to be promising. The following may be considered in future work:

   a. Systematic change in different process and formulation parameters for a better understanding of the mechanism of formation of hydrogel particles.

   b. Investigation of the kinetics of crosslinking reactions at different conditions on the gelation time, film thickness, and diffusion-controlled nature of these reactions.

   c. Study of the morphology of hydrogel particles and their water absorption capacity using SEM at different conditions. It is expected that this method produces particles with large voids (macroporous internal structure) and enhanced water absorption.

   d. A disadvantage of the proposed method is the need to remove the possible trapped oil from the particles. Light centrifugation of the mixture followed by a washing step with solvents is suggested as a method to fully separate the oil from the particles.

NOMENCLATURE

C	Salt concentration in the external phase
C_0	Initial salt concentration in the primary phase
CPI	Catastrophic phase inversion
D	Direct emulsification
$D_{3,2}$	Sauter mean diameter
EE	Encapsulation efficiency of multiple emulsions
f_d	Effective dispersed phase fraction
f_w	Fraction of water in emulsion
$H_{h,i}$	Contributions of the hydrophilic parts of the surfactant
$H_{l,i}$	Contributions of the hydrophobic parts of the surfactant
HLB	Hydrophilic-lipophilic balance
K	Conductivity of the emulsion
K_C	Conductivity of the continuous phase
K_d	Conductivity of the dispersed phase
K_w	Conductivity of external water phase in multiple emulsions
$K_{w,0}$	Conductivity of water phase in the primary emulsion
M_H	Molecular mass of the hydrophilic part of the surfactant
M_T	Molecular mass of the entire surfactant molecule
N_1	Stirring speed of the primary emulsification
N_2	Stirring speed of the secondary emulsification
O	Amount of oil
O/W	Oil-in-water
O/W/O	Oil-in-water-in-oil
PIT	Phase inversion temperature
S_1	Span 80 for the primary emulsification
S_2	Span 80 for the secondary emulsification
SW	Surfactant in water phase
SO	Surfactant in oil phase
TPI	Transitional phase inversion
W_1	Amount of water in the primary emulsion
W_2	Amount of water in the secondary emulsion
W/O	Water-in-oil
W/O/W	Water-in-oil-in-water
Greek letters	
ϕ	Internal phase fraction
ϕ_2	Phase fraction of multiple emulsion
$\phi_{1,0}$	Initial phase fraction of the primary emulsion
$\phi_{2,0}$	Initial phase fraction of the secondary emulsion
ϕ_w	Water phase fraction of multiple emulsion
ϕ_{2min}	Minimum phase fraction of multiple emulsion

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APPENDICES

7.1. Appendix A: Experimental Reproducibility

Several experiments were repeated throughout this work to verify the reliability of the experimental methods. For example, Figure 7.1 shows the conductivity measurements for two CPI emulsification experiments with exactly the same conditions and formulation (EXP_1 and EXP_2).

The error percentage in inversion time and drop size (measured by Master sizer) for these experiments are given in Table 7.1.

All experimental errors laid between 1-5.3% for drop size, and 5-10% for phase inversion point. Considering the sensitivity of conductivity measurements in an emulsion with catastrophic changes in drop size and morphology, the range of errors indicate very good reproducibility.

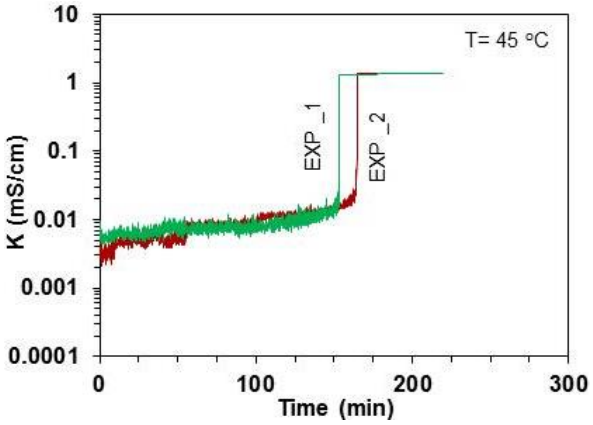

Figure 7.1. Time evolution versus conductivity for CPI (SO), [HLB = 14, [CO-720] = 5.0 wt.%, (f_w) = 0.25, T = 45ºC, stirring speed = 500 rpm, [KCl] = 0.5 wt.%].

Table 7.1. Error percentage for the emulsion drop size for CPI (SO), [HLB = 14, [CO-720] = 5.0 wt.%, (f_w) = 0.25, T = 45ºC, stirring speed = 500 rpm, [KCl] = 0.5 wt.%].

Parameter/Experiment	EXP_1	EXP_2	Error %
Phase inversion time (min)	155	163	5.23
$D_{3,2}$ (µm)	4.76	5.00	5.21

7.2. Appendix B: Optimum salt concentration in multiple emulsions

Prior to the study of effects of process variables on properties of multiple emulsions, we studied the effect of NaCl concentration in inner aqueous phase on the stability of W/O/W multiple emulsions.

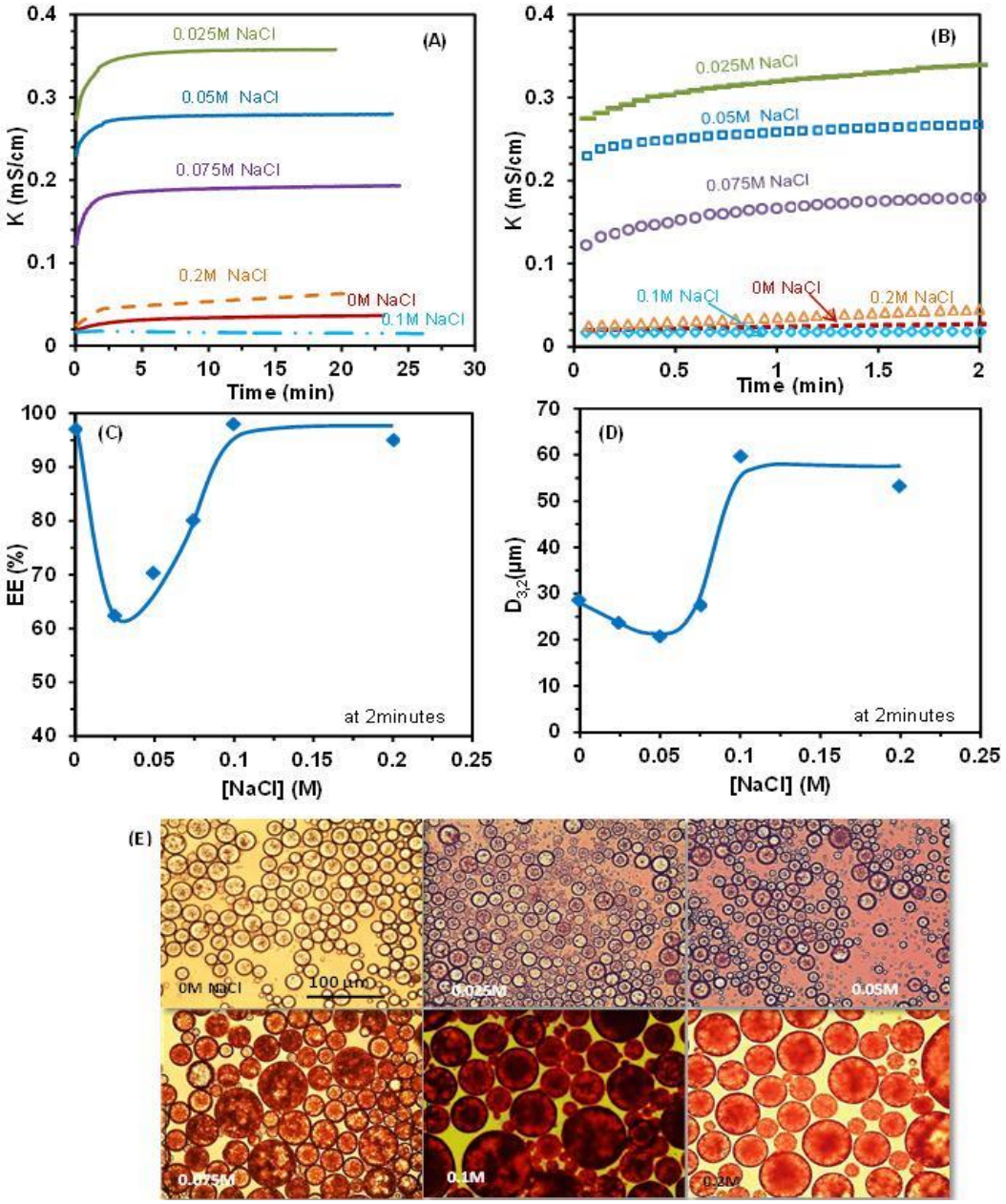

Figure 7.2. (A) Conductivity of multiple emulsions during and after emulsification, B) Conductivity of multiple emulsions during emulsification versus time at different versus time at different [NaCl], C) Encapsulation efficiency, D) $D_{3,2}$, E) Corresponding Micrographs of multiple emulsions ($Ø_{1,0}$= 0.30, $Ø_{2,0}$=0.30, N_1= 9400 rpm, N_2= 3400 rpm, [S_1] = 5%, [S_2] = 2%).

Figure 7.2(A) and (B) shows that the conductivity of multiple emulsions during and after secondary emulsification increases with time at low NaCl concentrations (< 0.1 M), but not significantly changed at higher NaCl

concentrations (≥ 0.1 M). This is due to the stabilising effects of the salt on multiple drops. Interestingly, having no salt in the inner aqueous phase also leads to a stable multiple emulsion; see also Figure 7.2(C), which shows that encapsulation efficiency increase as NaCl concentration increases. These results are broadly consistent with other studies, which have shown that release is reduced as the concentration of salt in inner phase increases (Magdassi and Garti, 1984;Hattrem et al., 2014). The existence and the concentration of electrolytes have a significant effect on the stability of W/O and W/O/W emulsions by balancing the Laplace pressure differences between the inner water droplets, and by playing a critical role in balancing osmotic pressure effects between the two water phases (Rosano et al., 1998). Using very high concentrations of electrolytes results in water migration from the external phase to the inner phase and later swelling of the W/O droplets releasing the internal material (Rosano et al., 1998). The highest NaCl concentration used in this study (0.2 M) did not seem to adversely affect the stability of multiple emulsion.

Figure 7.2(D) shows that the drop size is first decreasing at low salt concentrations, but then starts increasing showing enhanced stability.

Typical micrographs of multiple emulsions versus NaCl concentration are displayed in Figure 7.2(E). These micrographs are in agreement with the data collected for the drop size. They show a maximum encapsulation and maximum drop size at 0.1 M of NaCl. It can be concluded from the data collected in Figure 7.2(C), Figure 7.2(D) and Figure 7.2(E) that both drop size and encapsulation efficiency had their maximum values at 0.1M of NaCl meaning higher encapsulation efficiency. For this reason, 0.1M of NaCl is considered as the optimum concentration to study the effect of other parameters in this research.

Using 0.1M of NaCl in the inner aqueous phase provides maximum encapsulation efficiency under the conditions used in this study.

7.3. Appendix C: Research Outputs

1. Journal manuscripts (close to submission)

- Jahanzad, F., **Al-qutbi, A.**, and Sajjadi, S. "Agitation Induced Catastrophic Phase Inversion Emulsification; I. A Comprehensive Study", Will be submitted to *Colloids and Surfaces A: Physicochemical Engineering Aspects.*
- Jahanzad, F., **Al-qutbi, A.**, and Sajjadi, S. "Agitation Induced Catastrophic Phase Inversion Emulsification; II. Effects of Agitation Speed", Will be submitted to *Colloids and Surfaces A: Physicochemical Engineering Aspects.*
- Jahanzad, F., **Al-qutbi, A.**, Sugeevan, M., and Sajjadi, S. "Process Optimisation for Maximum Encapsulation During Formation of Multiple Emulsions", Will be submitted to *Journal of Colloid and Interface Science*.

2. Conference Contributions

- **Al-qutbi, A.**, Jahanzad, F. and Sajjadi, S. (2016) "Optimum Formulation for Maximum Encapsulation in Multiple Emulsions", *Formulla VIII*, Barcelona, Spain.
- **Al-qutbi, A.**, Jahanzad, F. and Sajjadi, S. (2015) "Dynamics of Catastrophic Phase Inversion Emulsification", *ChemEngDayUK*, University of Sheffield, UK.
- **Al-qutbi, A.**, Jahanzad, F. and Sajjadi, S. (2014) "Comprehensive Study of Catastrophic Phase Inversion", *International Colloid and Interface Science Symposium*, UK.
- **Al-qutbi, A.**, Jahanzad, F. and Sajjadi, S. (2014) "Catastrophic Phase Inversion vs. Direct Emulsification", *ChemEngDayUK*, University of Manchester, UK.

7.4. Appendix D: Risk Assessments Forms.

The risk assessment forms for all the equipment and chemicals used in this research are attached to the thesis.

Risk Assessment

General Health and Safety Risk Assessment Guidance

Risk Assessment is a process by which the risks of work activities are assessed and controlled. Both the Health and Safety at Work Act and the Management of Health and Safety at Work Regulations require the employer to produce risk assessments.

This document is intended to remove some of the burden of risk assessment by providing a structure for the process. The task of risk assessment may be delegated to a person who possesses the necessary skills to carry it out, however, Heads of Department remain responsible for the findings and implementation of the assessment.

In order to carry out a risk assessment the terms “risk” and “hazard” must be understood. Hazard is the potential to do harm and a risk in the chance of that hazard being realised. In order to prevent foreseeable risks occurring adequate controls must be put in place. The process is as follows;

- Heads of Department must identify the foreseeable hazards posed by work activities, working environment and the materials used.
- Identify who and how many people will be affected. Consider the experience, age, disabilities, medical condition and expertise of those involved.
- The location of the work be identified and stated.
- Adequate controls must be put into place and implemented under the authority of the Head of Department to prevent or reduce risk to individuals.
- The assessment must be signed and dated by the person responsible for generating the risk and a review date agreed. This safe system of work will need to be monitored to ensure that controls are effective.
- The amount of time put into a risk assessment should be proportional to the degree of risk.

An assessment should be carried out for each task e.g. moving chairs to a different location, using dangerous substances or machinery. Risk assessments

are working documents and should remain available to people to consult in the course of their everyday activities.

Risk Assessment Form

Task; Clearly identify the work covered by the assessment. Include a brief description of the work involved.

Creating Oil -in- water emulsions

- Surfactant will be dissolved in the oil phase (cyclohexane) and KCl will be dissolved in the water phase.
- KCl solution will be added to the cyclohexane.
- The mixture then will be stirrered to form emulsion with a mechanical stirring.
- Conductivity will be measured using conductivity meter.
- Droplets will be viewed using optical microscope.
- Droplets are measured using master sizer laser diffraction 2000.

Different conditions may be used. Temperature range (25°C-65°C).
Stirring speed range (500 rpm- 700 rpm).

Location; Where the task is to be carried out. Where this includes multiple locations these must also be stated.

- In lab E217 (Experimental work)

People involved; Individual names or if larger group are involved a general description. (e.g. staff, students, visitors, contractors or specific individuals.)

- Aseel al-qutbi

Hazards; A list of all those substances or actions which are hazardous. (e.g. working at computers, laboratory work, electricity, manual handling etc.)
Hazards: Tripping over, chemical spillage, bottles slipping while carrying. Harm by contact with skin/ eyes, through inhalation and if swallowed.

Risk Assessment; An assessment of the likelihood of injury resulting from the hazards.

- Low risk of surfactant and cyclohexane if the substances come in contact with skin inhaled or swallowed. This is avoided by using proper protective equipment while conducting the experiments. Protective equipment used will include: safety goggles, gloves, lab coat, separate bin for chlorinated waste and carriers to carry out the chemical bottles to avoid spillage.

The experiments will be conducted in a fume cupboard including weighing the chemicals.

Controls Measures; Put in place to prevent the risk occurring.(e.g. training, supervision, the use of less toxic materials, the use of guards, regular maintenance, providing more space etc.)
Personal Precautions: All glassware must be inspected before and after use. Emulsification is being conducted in a fume cupboard. Breathing vapours is avoided by adequate ventilation. Personal protection equipment is used while handling the chemicals.
Chemicals will be handled with a safety practice.
Storage Precautions: Chemicals will be stored in a cool place (E217). Containers will be tightly closed in a dry and well ventilated place. Bottles will be kept upright to prevent any leakage. Chemicals will be stored in designated cupboard and away from sources of ignition.
Spillage and Disposal: the chemicals are handled inside the fume cupboard at all time so in case of spillage, the fume cupboard will be closed. Chemicals will not be let down the drain. Separate individual bin for unchlorinated waste inside the fume cupboard will

be used. Carriers to carry out the chemical bottles to avoid spillage.

- This risk assessment will be reviewed in May, July and then on request.
- Also, the student will seek assistance from lab technicians if required.
- Minimal quantities on the chemicals are used.
- In case of any dizziness, the student will leave the laboratory and seek assistance.
- The student will contact her supervisor and health and safety officer if there are any concerns regarding safety in the laboratory.

Residual Risk; This is the risk remaining after the controls above are applied
The likelihood of any incidents occurring is reduced to a minimum by putting in place the listed control measures mentioned earlier and thus the overall risk is reduced.

I, the undersigned state that there is **no significant risk / the risks will be controlled by the methods stated on this form** (delete as appropriate). This assessment will be reviewed in one year, if the work should change or an unforeseen hazard arises.
Name:…….…… Date;…….
Signed;……. Position;……..

Risk Assessment Form

Task; Clearly identify the work covered by the assessment. Include a brief description of the work involved.

Creating water-in-oil -in- water emulsions

Multiple emulsions are prepared using a two-step emulsification method. The first step is to make the primary, W/O, emulsion.

- ❖ 0.1 M of NaCl is dissolved in the water phase
- ❖ The aqueous solution is introduced into the oil phase (paraffin).
- ❖ A given amount of Span 80 is dissolved in the oil prior to the addition of the aqueous solution.
- ❖ The emulsion is homogenised for 2 min at a given stirring speed using Eurostar digital stirrer.
- ❖ In the second step, the primary emulsion is dispersed into another continuous water phase containing a given amount of Tween 80.
- ❖ The secondary emulsification will be carried out using the same stirrer for 2 min at a given stirring speed.
- ❖ All experiments will be performed at room temperature.
- ❖ Conductivity will be measured using conductivity meter.
- ❖ Droplets will be viewed using optical microscope.
- ❖ Droplets are measured using master sizer laser diffraction 2000.

Location; Where the task is to be carried out. Where this includes multiple locations these must also be stated.

- ❖ In lab E217 (Experimental work).

People involved; Individual names or if larger group are involved a general description. (e.g. staff, students, visitors, contractors or specific individuals.)

- ❖ Aseel al-qutbi

Hazards; A list of all those substances or actions which are hazardous. (e.g. working at computers, laboratory work, electricity, manual handling etc.)

Hazards: Tripping over, chemical spillage, bottles slipping while carrying. Harm by contact with skin/ eyes, through inhalation and if swallowed.

Risk Assessment; An assessment of the likelihood of injury resulting from the hazards.

- Low risk of surfactant and cyclohexane if the substances come in contact with skin, inhaled or swallowed. This is avoided by using proper protective equipment while conducting the experiments. Protective equipment used will include: safety goggles (EN166), gloves resistant to solvents (EN374), , lab coat, separate bin for chlorinated waste and carriers to carry out the chemical bottles to avoid spillage.

The experiments will be conducted in a fume cupboard including weighing the chemicals.

Controls Measures; Put in place to prevent the risk occurring. (e.g. training, supervision, the use of less toxic materials, the use of guards, regular maintenance, providing more space etc.)

Personal Precautions: All glassware must be inspected before and after use. Emulsification is being conducted in a fume cupboard. Breathing vapours is avoided by adequate ventilation. Personal protection equipment is used while handling the chemicals.

Chemicals will be handled with a safety practice.

Storage Precautions: Chemicals will be stored in a cool place (E217). Containers will be tightly closed in a dry and well ventilated place. Bottles will be kept upright to prevent any leakage. Chemicals will be stored in designated cupboard and away from sources of ignition.

Spillage and Disposal: the chemicals are handled inside the fume cupboard at all time so in case of spillage, the fume cupboard will be closed. Chemicals will not be let down the drain. Separate individual bin for unchlorinated waste inside the fume cupboard will be used. Carriers to carry out the chemical bottles to avoid spillage.

- This risk assessment will be reviewed in May, July and then on request.
- Also, the student will seek assistance from lab technicians if required.
- Minimal quantities on the chemicals are used.
- In case of any dizziness, the student will leave the laboratory and seek assistance.
- The student will contact her supervisor and health and safety officer if there are any concerns regarding safety in the laboratory.

Residual Risk; This is the risk remaining after the controls above are applied

The likelihood of any incidents occurring is reduced to a minimum by putting in place the listed control measures mentioned earlier and thus the overall risk is reduced.

I, the undersigned state that there is **no significant risk / the risks will be controlled by the methods stated on this form** (delete as appropriate). This assessment will be reviewed in one year, if the work should change or an unforeseen hazard arises.

Name:…… Date;…….

Signed;…… Position;……..

Risk Assessment Form

Task; Clearly identify the work covered by the assessment. Include a brief description of the work involved.

Synthesising hydrogel particles using two steps.

- **In the first step**, the normal NaAlg -in-oil emulsion (W/O) will be prepared in a jacketed glass reactor. The alginate solution will be prepared by dispersing sodium alginate in distilled water. The solution will be stirred for two hours at 500 rpm using a mechanical stirrer. Then alginate solution will first introduced to the oil phase containing Span 20. Mixing speed is 500 rpm using Eurostar digital stirrer.
- **While in the second step**, a normal $CaCl_2$ -in-oil emulsion will be prepared in a small beaker. $CaCl_2$ solution will be introduced to the oil phase containing Span 20, and the two phases will be mixed using homogeniser (3200 rpm) for 2 minutes.
- Then the $CaCl_2$ emulsion will be added to the NaAlg emulsion, mixing speed will be kept 500 rpm.
- Then the particles will be washed to remove the excess of the oil.
- Finally, the particles will be left to dry at in the oven at 50◦C for 6 - 8 hours.
- All experiments will be conducted at 25 ^{o}C.
- Conductivity will be measured using conductivity meter.
- Particles will be viewed using optical microscope.

Location; Where the task is to be carried out. Where this includes multiple locations these must also be stated.

- In lab E217 (Experimental work).

People involved; Individual names or if larger group are involved a general description. (e.g. staff, students, visitors, contractors or specific individuals.)

- Aseel al-qutbi

Hazards; A list of all those substances or actions which are hazardous. (e.g. working at computers, laboratory work, electricity, manual handling etc.)

Hazards: Tripping over, chemical spillage, bottles slipping while carrying. Harm by contact with skin/ eyes, through inhalation and if swallowed.

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The experiments will be conducted in a fume cupboard including weighing the chemicals.

Controls Measures; Put in place to prevent the risk occurring.(e.g. training, supervision, the use of less toxic materials, the use of guards, regular maintenance, providing more space etc.)

Personal Precautions: All glassware must be inspected before and after use. Emulsification is being conducted in a fume cupboard. Breathing vapours is avoided by adequate ventilation. Personal protection equipment is used while handling the chemicals.

Chemicals will be handled with a safety practice.

Storage Precautions: Chemicals will be stored in a cool place (E217). Containers will be tightly closed in a dry and well ventilated place. Bottles will be kept upright to prevent any leakage. Chemicals will be stored in designated cupboard and away from sources of ignition.

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- This risk assessment will be reviewed in May, July and then on request.
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Name:…… Date;…….

Signed;…… Position;……..

7.5. Appendix E: Postgraduate key skills development

1. I have attended the following training sessions which organised by LSBU, the certificates of attendance are attached to the thesis.

- Key Skills in the Research Environment - 5 November 2012.
- The Student-Supervisor Relationship - 07November 2012.
- Personal Effectiveness - 09 November 2012.
- Conference Presentation - 12 November 2012.
- Thesis Submission, Viva and Career Management - 25 April 2013.
- Academic Publication - 26 April 2013.
- Thesis Submission and Viva - 26 June 2013.
- Next Steps: After Your Research Degree (Looking Ahead) - 26 June 2013.
- Intellectual Property – 25 February 2014.

2. I also have been awarded PGCHE (the certificate is attached to the thesis).

3. ***Additional Training Sessions:***

- Health and Safety Induction - 8 June 2012.
- Career Management - 25 April 2013.
- Introduction to the Moodle Virtual Learning Environment - 8 May 2014.
- Introduction to Grant Writing - 30 June 2017.
- For Successful Doctorate - 13 June 2017.
- Haplo PGR Manager (Postgraduate research student management system) - 13 July 2017.

London South Bank University

PG Certificate in Research Skills

Postgraduate Training Session

CERTIFICATE OF ATTENDANCE

THIS IS TO CERTIFY THAT

ASEEL AL-QUTBI

ATTENDED A COURSE OF INSTRUCTION

The Student–Supervisor Relationship

ON 7 NOVEMBER 2012

PROFESSOR PETER DOYLE
PG CERTIFICATE COORDINATOR
LONDON SOUTH BANK UNIVERSITY

London South Bank University

PG Certificate in Research Skills

Postgraduate Training Session

CERTIFICATE OF ATTENDANCE

THIS IS TO CERTIFY THAT

ASEEL AL-QUTBI

ATTENDED A COURSE OF INSTRUCTION

Key Skills in the Research Environment

ON 5 NOVEMBER 2012

PROFESSOR PETER DOYLE
PG CERTIFICATE COORDINATOR
LONDON SOUTH BANK UNIVERSITY

London South Bank University

PG Certificate in Research Skills

Postgraduate Training Session

CERTIFICATE OF ATTENDANCE

THIS IS TO CERTIFY THAT

ASEEL AL-QUTBI

ATTENDED A COURSE OF INSTRUCTION

Conference Presentation

ON 12 NOVEMBER 2012

PROFESSOR PETER DOYLE
PG CERTIFICATE COORDINATOR
LONDON SOUTH BANK UNIVERSITY

London South Bank University

PG Certificate in Research Skills

Postgraduate Training Session

CERTIFICATE OF ATTENDANCE

THIS IS TO CERTIFY THAT

ASEEL AL-QUTBI

ATTENDED A COURSE OF INSTRUCTION

Personal Effectiveness

ON 9 NOVEMBER 2012

PROFESSOR PETER DOYLE
PG CERTIFICATE COORDINATOR
LONDON SOUTH BANK UNIVERSITY

London South Bank University

Postgraduate Certificate in Research Skills
Key Skills Development Programme Training Session

CERTIFICATE OF ATTENDANCE

THIS IS TO CERTIFY THAT

ASEEL AL-QUTBI

ATTENDED A COURSE OF INSTRUCTION

Academic Publication

ON 26 APRIL 2013

PROFESSOR PETER DOYLE
POSTGRADUATE SKILLS COORDINATOR
LONDON SOUTH BANK UNIVERSITY

London South Bank University

Postgraduate Certificate in Research Skills
Key Skills Development Programme Training Session

CERTIFICATE OF ATTENDANCE

THIS IS TO CERTIFY THAT

ASEEL AL-QUTBI

ATTENDED A COURSE OF INSTRUCTION

Thesis Submission, Viva and Career Management

ON 25 APRIL 2013

PROFESSOR PETER DOYLE
POSTGRADUATE SKILLS COORDINATOR
LONDON SOUTH BANK UNIVERSITY

London South Bank University

Postgraduate Certificate in Research Skills
Key Skills Development Programme Training Session

CERTIFICATE OF ATTENDANCE

THIS IS TO CERTIFY THAT

ASEEL AL-QUTBI

ATTENDED A COURSE OF INSTRUCTION

Thesis Submission and Viva

ON 26 JUNE 2013

PROFESSOR PETER DOYLE
POSTGRADUATE SKILLS COORDINATOR
LONDON SOUTH BANK UNIVERSITY

London South Bank University

Postgraduate Certificate in Research Skills
Key Skills Development Programme Training Session

CERTIFICATE OF ATTENDANCE

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ATTENDED A COURSE OF INSTRUCTION

Next Steps: After Your Research Degree – Looking Ahead

ON 26 JUNE 2013

PROFESSOR PETER DOYLE
POSTGRADUATE SKILLS COORDINATOR
LONDON SOUTH BANK UNIVERSITY

London South Bank University

RESEARCHER DEVELOPMENT

CERTIFICATE OF ATTENDANCE

THIS IS TO CERTIFY THAT

Aseel AL-QUTBI

ATTENDED A COURSE OF INSTRUCTION

Intellectual Property

ON 25 FEBRUARY 2014

CHUNG LAM
RESEARCH SUPPORT OFFICER

Certificate

This is to certify that
Aseel Al Qutbi

has achieved the status of
Fellow
of The Higher Education Academy

in recognition of attainment against the
UK Professional Standards Framework for
teaching and learning support in higher education.

Recognition reference:
PR088864
Date of recognition
09/07/2015

Professor Stephanie Marshall
Chief Executive
The Higher Education Academy

Professor Don Nutbeam
Chairman of the Board of Directors
The Higher Education Academy Board